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PATHOLOGICAL CHANGES ACCOMPANYING INJECTIONS OF AN ACTIVE DEPOSIT OF RADIUM EMANATION

I. INTRAVENOUS AND SUBCUTANEOUS INJECTIONS IN THE WHITE RAT

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Very little is known concerning the changes that occur in living tissue following the injection of solutions of an "active deposit" of radium emanation. This investigation was undertaken to determine what changes occur in the principal organs of the animal body following such a treatment, and to afford data that might act as a guide in the treatment of certain types of cancer, in which the solution method of radium therapy appeared particularly adapted. Since the main facts of this investigation became apparent, cases of leukemia and lymphosarcoma have been treated by the methods employed in these experiments. In these diseases the lesions were so widely disseminated as to suggest treatment by the method of intravenous radium injection.

The scope of the investigation may be divided into two parts, one dealing with experiments concerning intravenous, and the other with subcutaneous injections of the "active deposit" of radium emanation.

APPARATUS AND METHODS

At the Memorial Hospital the Duane type of radium emanation apparatus is used. It was devised by Doctor William Duane, and adequately described in The Boston Medical and Surgical Journal (1).

Instead of collecting the radium emanation by compressing it in small glass tubes, this being the usual method, the emanation for these experiments was collected in the form of what is called an "active deposit" upon common salt. A small quantity of salt was placed in the upper glass bulb, attached to a larger bulb as indicated in figure 1, and the bulbs were then sealed to the end of a radium emanation purifying apparatus. Then a considerable quantity of emanation, in the form of a gas, was forced into the bulb containing the salt. This was done by first creating a vacuum, and later forcing the purified emanation

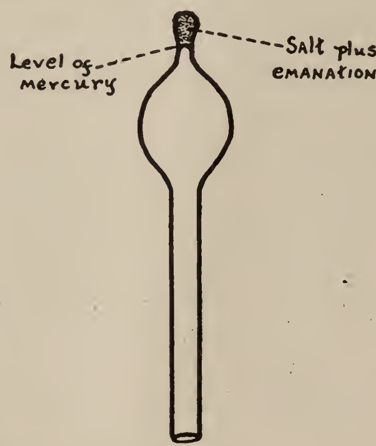

Fig. 1 Apparatus used in collecting active deposit of radium.

ahead of a column of mercury, until all the emanation was contained in the small bulb in which the salt had been placed. After about three hours the salt had received the maximum amount of radio-active deposit. The glass bulb was then cut from the apparatus, and the salt, containing the active deposit of radium, was dissolved in sufficient distilled water to bring the solution to the strength of a physiological salt solution. The liquid was drawn into a 2 cc. Luer syringe, which was covered by a lead shield as a protection for the fingers of the experimenter, and the syringe, needle, and solution were measured in an accurate electrometer. The activated solution exhibited all the known

phenomena of radium metal itself. The strength of the solution was determined in millicurie units. In this manner a considerable quantity of active radium substance was obtained in a relatively small amount of liquid. For example, the usual amount collected was about 150 mc. in 2 cc .of solution.

This method of collecting radium deposit was found to be very economical. Only a little of the main supply of the hospital's emanation was actually used, since only a small part, 2 to 3 per cent, of the emanation had actually been given off as a deposit on the salt, and after the three hours exposure the remaining emanation was removed and collected in glass tubes for routine therapeutic use.

In the solution used in these experiments the radiation consisted of alpha, beta, and gamma rays, but the greatest physiological effects produced in the tissues were probably the result of alpha ray activity. (For a further consideration of the physical properties of radium emanation see references (2), (3) and (4).)

Following the well known physical phenomenon, the radioactivity, after reaching its maximum strength in three hours, decayed at a uniform rate, and so it was necessary to dissolve the salt, measure the syringe and needle, and inject the solution into the animals with as little loss of time as possible. However, in all cases a larger amount of radium was collected than was needed at one time, so that one might take advantage of the radium decay by waiting until the solution had reached a desired dosage before the injections were made. If preparations are made in advance for quickly injecting the solution, only a relatively small amount of the radio-activity is deposited upon the walls of the syringe and the needle. After the injection is made the empty syringe and needle are at once measured in an electrometer, so that the amount of radio-activity left in the apparatus may be determined. When this is subtracted from the previous figure, obtained for the syringe plus the solution, the amount of radium that was actually contained in the solution may be calculated. After a little experience it is possible to approximate very nearly a required dose. All the doses were given in millicurie units of "active deposit" of radium

emanation as described above, and if reference is later made to the injected solution as, radio-active substance, activated solution, etc., be it understood that these terms are used interchangeably with "active deposit."

Only healthy, three-quarters to full grown, rats were used in this investigation. They varied in weight from 200 to 250 grams. The injections were made under light ether anesthesia. Caudal vein injections were made in the intravenous series, while in the subcutaneous groups the solutions were deposited under the skin in the right shoulder region.

Some of the animals were killed by the experimenter for histological study. These animals were put under ether anesthesia and the organs quickly removed and placed in fixative.

EXPERIMENTAL RESULTS

Series A. Intravenous injections

In this series twelve animals were used, and caudal injections were given in doses ranging from 2.6 mc. to as high as 135 mc. each.

Table 1 gives in the first two columns, the serial number for each animal, and the dose employed in millicuries. In the third column is indicated the time when the animals died, apparently as a result of the treatment. The last column indicates the day on which the animals that received the smaller doses were killed by the experimenter for histological study. We find in table 1, that doses ranging from the smallest, 2.6 mc., to 10.6 mc., were not sufficient to cause the death of the animals within a month's time after the injection. From this group of animals that survived the treatment for a considerable period, one was killed on the thirty-fifth and another on the thirty-seventh day after treatment. Two were killed on the forty-fourth and two on the forty-fifth day, and finally one was killed on the seventy-eighth day after treatment. The table indicates however, that an increase from 10.6 mc. to 11.2 mc. proved fatal in the case of animal VIII. This animal died within two and a half days after treatment. Likewise, all the doses above 11.2 mc. resulted

in immediate, acute effects. Animal IX, after receiving an intravenous injection of 19.8 mc., was so ill at the end of four days that it would soon have died as a result of the treatment, had it not been killed by the experimenter in order that its organs might be preserved in the most favorable condition for histological study. Animal X received a dose of 27.8 mc., and died at the end of three days. In like manner animal XI, that received 29.6 mc., after showing very severe symptoms, died at the end of the third day. Animal XII, after receiving 135 mc.,

TABLE 1

Series A. Results for the intravenous injections of solutions of "the active deposit" of radium emanation

ANIMAL NUMBER	NUMBER OF MILLICURIES OF RADIUM EMANATION	DAY ON WHICH ANIMAL DIED AS A RESULT OF THE TREATMENT	THE DAY FOLLOWING THE TREATMENT ON WHICH THE ANIMAL WAS KILLED FOR AUTOPSY
I	2.6		45
II	2.9		78
III	4.3		45
IV	5.7		44
V	7.1		35
VI	8.8		37
VII	10.6		44
VIII	11.2	2.5	
IX	19.8	*	
X	27.8	3	
XI	29.6	3	
XII	135.0	†	

* Was very ill at the end of the fourth day and was then killed.

† Died a few hours after the treatment, between 5 p.m. and 9 a.m.

the largest dose of radium that was used in any one instance, died a few hours after the injection.

The experimental data that follow give the histological reports for the twelve animals that received radium treatment by the intravenous method. The organs showing the greatest pathological changes were, the liver, the lungs, the kidneys, and the spleen. The bone marrow and adrenals were considerably altered in certain cases, and changes occurring in the vascular supply of the brain were noted. No data were obtained for the changes that occurred in the blood following the treatments as

it was decided to test this point on a larger type of animal. This is now being done, and the results will be published at a later time.

The experiments are presented according to the size of the dose employed. From the standpoint of the general physiological reactions, animals I to VII inclusive may be said to show more or less chronic effects, while animals VIII to XII, that received the larger doses, exhibited decidedly acute reactions. Animal III, for example, was treated on June 10, 1918, with an injection of 4.3 mc., and was killed by the experimenter forty-five days later. This was one of the smaller doses, for, as may be seen in table 1, animal VII was treated with as large a dose as 10.6 mc. and still lived until it was killed forty-four days later. And yet the pathological findings for animal III showed an intense congestion of the liver, with severe acute degeneration of a fatty granular nature. The kidneys were also intensely congested and hemorrhages occurred in the glomeruli, cortex, and medulla. A condition of purulent bronchitis and broncho-pneumonia was found in the lungs, while the spleen was intensely congested and hemorrhagic, with reduction in size of the follicles.

It was found, that even with a still smaller dose, such as 2.6 mc., which was given to anmal I, there was produced in the kidneys an intense acute congestion, edema of the lungs, and also reduction in size of the lymph follicles in the spleen. In the last two organs, however, the small dose of radium produced no hemorrhages, and it is well to note here that with larger doses congestion associated with hemorrhages occurred in these organs almost constantly.

Animal IV was treated with 5.7 mc. of radium, and, although the liver of this animal showed some congestion, still this condition was less marked than was found for the larger doses. There was but little congestion in the kidneys, and the lungs were in a fairly good condition. In this case the spleen appeared considerably affected. It was intensely congested, and this condition was almost as pronounced as that which occurred in the spleen of animals that had received very large doses.

With a somewhat larger dose of radium, 7.1 mc., animal V presented changes in the liver similar to those of animal IV; but in the case of animal V, the kidneys and lungs showed intense congestion, while in the spleen no appreciable congestion was found. In the case of animal VI, that received a somewhat larger dose, 8.8 mc., and was killed by the experimenter thirty-seven days after the treatment, the principal conditions noted in the organs were: a cloudy swelling of the liver cells, with advanced parenchymatous hepatitis; congestion of the kidneys, adrenals, spleen, and lungs, and also in the last named organ a perivascular edema.

The largest dose of radium that was survived for a considerable length of time, occurred in animal VII. This animal received 10.6 mc. and was killed, for purposes of autopsy, on the forty-fourth day. The liver was congested, but the condition in this case was not as marked as in the animals that received the still larger doses. The kidneys were in a state of severe granular degeneration, with intense congestion, while the swelling of the tufts of the glomeruli filled the capsules completely, but there was no cell necrosis. The lung lesion was an acute bronchitis, associated with areas of bronchopneumonia. The spleen showed intense hyperplasia of the lymph follicles, and some congestion.

As we go from animal VII to animal VIII, although the increase in dose was only 0.6 mc., we find in the latter case that the animal died two and a half days after its treatment. The dose in this case was 11.2 mc. and following it we find the occurrence of more active, acute conditions. The liver contained focal areas of necrosis, usually near the blood vessels and associated with hemorrhage. The kidneys were congested and hemorrhagic, and the lungs showed intense congestion, with hemorrhagic pneumonia. There was an edematous condition about the blood vessels and the bronchi. However, the spleen and also the brain, showed but relatively little congestion. Plate 1 shows the conditions that resulted in the kidney of this animal.

Following an injection of 19.8 mc. of radium, animal IX soon became very ill. It exhibited all the signs of an acute enteritis,

and it was killed by the experimenter on the fourth day after its treatment. The liver of this animal was intensely congested, and the kidneys showed hemorrhagic nephritis, with dilatation of the large blood vessels. The lungs in this case were also congested.

Animal X received an injection of 27.8 mc., and died as a result of the treatment three days later. As in the preceding case, the animal also exhibited severe symptoms of an intestinal disturbance. The histological data showed an intensely congested liver, especially of the capillaries. There was perivascular edema of the large blood vessels of the portal canals, and in some cases there appeared to be a beginning thrombosis and fibrinous deposit in the blood vessels. In the kidneys a severe, acute degeneration had taken place, which was associated with an intense congestion, involving to a marked extent, the glomeruli and the cortical tubules. The splenic follicles were prominent. The splenic pulp, the brain, and the lungs were intensely congested. In the lungs a partial destruction of the large veins was noted.

On January 10 animal XI received 29.6 mc. of radium. On January 12 it appeared fairly normal, but on the next day it became very ill and died during the following night. The kidneys were congested, and a granular degeneration was present in the cells of the tubules. In some places this degeneration almost amounted to necrosis. In the lungs there was extreme venous and capillary congestion. This marked congestion had also extended to the spleen. The sinuses of that organ were choked with large phagocytic cells and old blood pigment, indicating a considerable red blood cell destruction. The follicles in the spleen were reduced in size. The bone marrow of the sternum was extremely congested. Few marrow cells remained, and in some places there were none at all. The marrow spaces were occupied by a deposit of diffuse blood. The fat cells were indistinct, and many appeared to be broken up into globules of various sizes.

The largest dose of radium administered at one time was given to animal XII. This animal received an intravenous injection

of 135 mc. It died during the night of the same day, between 5 p.m. and 9 a.m. Unfortunately the liver and spleen were destroyed by its cage mates, but satisfactory sections were obtained from the kidneys, adrenals, lungs, and bone marrow. The kidneys showed diffuse congestion, and there was enormous congestion in the adrenals. In the lungs the alveoli were filled with red blood cells. There was a marked perivascular and peribronchial edema, and there were also evidences of an interstitial bronchitis, with a marked desquamation of the epithelial cells of the bronchi.

A recapitulation and discussion of these results will be deferred until the following data for the second series, series B, are given.

TABLE 2

Series B. Results for the subcutaneous injections of the "active deposit" of radium emanation

ANIMAL NUMBER	NUMBER OF MILLICURIES OF RADIUM EMANATION	DAY ON WHICH ANIMAL DIED AS A RESULT OF THE TREATMENT	THE DAY FOLLOWING THE TREATMENT ON WHICH THE ANIMAL WAS KILLED FOR AUTOPSY
I	3.5		64
II	3.5		64
III	7.0		64
IV	9.1		6
V	9.1		4
VI	17.0	5	
VII	17.0	4	
VIII	19.0		7
IX	19.0	6	
X	20.5	5	

SERIES B. SUBCUTANEOUS INJECTIONS

In table 2 are given the results obtained from the series of subcutaneous injections. These injections were made under the skin in the right shoulder region. The method of arranging the data is the same as in the first table. Comparing the two sets of results we find them similar in regard to the lethal effects that were produced in the animals. Here it is seen that doses up to about 10 mc. are not fatal. In this series a subcutaneous injection of 17 mc. killed an animal in five days. It is possible that

the destructive effects produced from subcutaneous injections are less severe than in the case of the intravenous injections. The data suggest this conclusion, but are not sufficient to be conclusive. This point will be discussed later in the report.

Two animals received subcutaneous injections of 3.5 mc. each, and lived until they were killed by the experimenter sixty-four days after treatment. Another animal, that received 7.0 mc., was killed in the same manner on the sixty-fourth day. Two animals, each receiving 9.1 mc., were killed on the sixth and fourth day respectively. Two animals received doses of 17.0 mc. each, and they died, apparently as a result of the treatment, one on the fifth, and the other on the fourth day following the injections. Two more animals were injected and each received 19.0 mc. One died at the end of six days, the other was in a fairly good condition when killed by the experimenter. This animal, no. VIII of series B, was apparently exceptional in its resistance to the lethal effects of radium. Its organs suffered definite lesions, however, which will be described later. The tenth, and last animal of the series, was killed in five days by a dose of 20.5 mc. It exhibited symptoms of severe intestinal disturbance.

In the data that follow for the series of subcutaneous injections, it will be noted that, as in the case of the intravenous injections, the liver and kidney suffer largely as a result of exposure to radium emanation. However, probably due to the relatively slower rate of diffusion throughout the body in the case of the subcutaneous method, the lungs in this series are very much less affected. Only three out of ten animals that received subcutaneous injections gave definite lung lesions, while the lungs of the remaining seven animals were apparently normal. This condition held even after comparatively large doses.

It was also noted that the local reaction of the subcutaneous tissues in the region of the point of injection was slight. This is probably due to the fairly rapid manner in which the solution was diffused in the tissues of the shoulder region. In none of the cases was necrosis produced. In some of the early caudal vein injections, however, a small part of the radium was depos-

ited outside the blood vessel, and in such cases a small necrotic area was produced at the point of injection. This condition resulted because the diffusion was slow, and the radio-activity was deposited locally.

Animals I and II received subcutaneous injections of radium amounting to 3.5 mc. each. They were killed by the experimenter sixty-four days after the treatment. The liver in both cases showed congestion and fatty degeneration, with the presence of many giant cells, and numerous hyperchromatic nuclei. In each case the lungs were normal, while the bone marrow and spleen of animal I showed no definite lesions, yet the kidneys of this animal were much congested. The renal tubules appeared normal, but there was hyperchromatism of the cells of the glomeruli. In animal II the renal tubules were dilated and filled with coagulum, while in the cells there was a granular degeneration. The bone marrow was congested, but otherwise was little altered, while the spleen was severely congested and contained prominent follicles.

A single subject, animal III, was injected with 7.0 mc., and was killed sixty-four days later. In this animal the liver showed the greatest changes, exhibiting a very marked fatty degeneration, with the nuclei of many cells much enlarged and hyperchromatic. The kidney was normal, but for a certain amount of capillary congestion. The lungs, bone marrow, and spleen were normal, except that the spleen was small, and in the bone marrow there was some dilatation of the sinuses.

Two animals, nos. IV and V, were each given a subcutaneous injection of 9.1 mc. of radium. The former was killed on the sixth day and the latter on the fourth day following. Animal V showed but slight degeneration of the liver, but there was a multiplication of the nuclei of the cells of that organ. The liver of animal IV, however, showed fatty degeneration and was severely congested. The kidneys in both animals were congested, but showed only a slight degeneration. The lungs of animal V were normal, while in animal IV, bronchopneumonia and catarrhal bronchitis were present, and in the latter animal there was congestion of the bone marrow, and but few foci of the marrow cells remained.

Animals VI and VII were each given subcutaneous injections amounting to 17.0 mc. One died four days later, and the other five. Before death each exhibited symptoms of severe intestinal disturbances. Unfortunately the histological data are lacking, and for this reason approximately the same doses were repeated for two other animals, nos. VIII and IX. These latter each received a dose of 19.0 mc. Animal IX died on the sixth day, but animal VIII proved particularly resistant and lived until it was killed on the seventh day. Although animal VIII lived for a week after its treatment, and was apparently in a fairly good condition before it died, still its organs proved to have suffered profound pathological changes. A severe granular and fatty degeneration occurred in the liver which was associated with much congestion, and the production of cells with large nuclei. In the kidneys there was congestion and granular degeneration, while the lungs contained a slight bronchopneumonia, with perivascular edema. There was not much congestion in the spleen, but an increase in size of the malpighian bodies was noted.

The following results were obtained for animal IX. Congestion and acute degeneration were present in the liver and kidneys, while in the lungs there was a marked bronchopneumonia. In the spleen great congestion had occurred, with damage to the splenic cells. The malpighian bodies were small, and the bone marrow was largely replaced by blood.

The largest subcutaneous injection was given to animal X. The animal received 20.5 mc., and died after five days. There was found in the liver a very severe congestion and degeneration of the hepatic cells, with the formation of many giant cells, with large nuclei, which were homogenous and hyperchromatic. The renal tubules were dilated, and a granular degeneration and erosion of the cells had taken place. The lungs were normal, but the bone marrow was replaced by blood, no marrow cells being retained. The conditions occurring in the liver of this animal are shown in plate 2.

SERIES C. CONTROL GROUP

A large series of sections was used as a control to these experiments. These sections were taken from normal untreated animals, killed in the same manner as the treated individuals, and also from animals injected intravenously and subcutaneously with salt not exposed to radium. A third control group was composed of animals injected with salt that had been exposed to a very large amount of radium emanation, and was then allowed to "decay," until the active deposit had lost its radio-activity. These animals came from the same stock that was used in series A and B, and were of about the same size, weight, etc., as the animals of the experimental groups.

The animals treated with ordinary salt, and those treated with salt that had been exposed to a large amount of radium emanation and then allowed to "decay," showed absolutely no symptoms of enteritis and appeared to be perfectly normal, as regards both their physical condition and the histological study of their organs.

DISCUSSION OF RESULTS

The results of this investigation show that injections of an active deposit of radium emanation, applied by the intravenous or the subcutaneous methods, bring about very definite changes in the animal body. It has also been found that sublethal doses, although they permit the animal to live for a fairly long time, result in pathological changes in the organs of a more or less chronic nature. It is noteworthy, in this regard, to refer to the changes that occur in the liver, especially those resulting from small doses of radium injected subcutaneously. Here we find a fatty degeneration, which, in the animals of this series, was very pronounced and frequent and was found to be present a long time after treatment. This condition was associated with the appearance of many giant cells and large nuclei, probably as a result of a regenerative process. It is interesting to note at this point that Mills (5) reported in 1910, a similar condition resulting from external applications of radium. He found that

by exposing a series of mice to gamma rays, and using for thirty minutes over the region of the liver, what amounted to about 25 mgm. of radium bromide, "a transient change in the liver cells, somewhat resembling a cloudy swelling," took place. In the present investigation, as previously stated, the changes in the tissues are probably due mainly to alpha ray activity, and so it would appear that the type of degeneration referred to above may result from one or from a combination of the three types of radiation given off by the radium.

Following comparatively large doses of radium the animals died in from a few hours to six days. The accompanying lesions were severe congestion, frequently associated with diffuse hemorrhages, in the liver, kidney, lung, spleen, and bone marrow. In the case of intravenous injections the lungs were found to be severely affected. The large doses of radium produced a proliferation and desquamation of the epithelial cells of the bronchi, and there was also an apparent rupture of the blood vessels themselves, so that the air spaces were filled with red blood cells. This condition was associated with marked edema about the blood vessels and the bronchi. A similar vascular response for the blood vessels of the skin, was observed by Thies (6), for, after exposing the skin of guinea-pigs for six hours to 20 mgm. of radium bromide, he noted a dilatation of the blood vessels, and the presence of capillary hemorrhages.

This investigation shows that in the kidneys the most frequent condition following, or accompanying congestion, was a granular degeneration and erosion of the renal cells.

The changes in the bone marrow resulted in its replacement by blood. In many cases it was found that following a radium injection no marrow cells remained. This observation confirms the work of Thies, obtained by irradiating white mice in toto.

Congestion of the spleen was found to be the most constant feature resulting from radium treatment. This was also found to be associated, in some cases, with hemorrhage and a destruction of red blood cells.

In certain cases, following fairly large doses of radium, the blood vessels of the brain were found to be intensely congested.

The small vessels were dilated and gorged with blood. This observation is in line with that of Danysz (7), who reported the presence of hemorrhages in the brain and spinal cord of mice exposed to radium.

It seems fairly evident from a comparison of the results of this investigation with those already obtained by other investigators, that the changes that occur in the animal tissues following an injection of a radio-active solution, either in the blood stream directly, or indirectly by the subcutaneous method, are apparently similar to the changes that follow the external application of radiations from radium bromide itself.

There have been some data accumulated concerning the elimination of radium after its injection in the animal body, and they are mentioned at this point as they probably have some bearing on the interpretation of the results of this study. To quote from Meyer (8), 1906–1907, who obtained his results by using solutions of radium bromide, "The liver, lungs, and kidneys appear to be among the first organs to show the presence of radium after its intravenous injection. The ultimate fate of radium introduced subcutaneously, intraperitoneally, or per os is not materially different from that of radium introduced intravenously."

Berg and Welker (9) reported, 1905–1906, that "after subcutaneous injections, radium (bromid), like barium, calcium, and similar elements, is eliminated per rectum. The intestine seems to be the main channel of radium excretion."

Salant and Meyer (10), 1907–1908, found that "the elimination of radium takes place chiefly through the liver, the kidneys and the small intestine, and to a lesser extent also, through the large intestine in some of the herbivora."

From some unpublished work of the present writer, it was found that if shortly following an intravenous injection of the "active deposit" of radium emanation, prepared as described in this article, the blood vessels of the large viscera were ligated, and the organs, plus the blood they contained, were then tested for their radio-activity, the liver showed the presence of the greatest amount of this activity. The activity found in the two kidneys together was about a third the amount found in the

liver. The stomach and intestinal tract contained slightly more radium than the kidneys, and the lungs slightly less than half the amount detected in the latter, while in the spleen there was a little less radium than was detected in the lungs. This work was performed on the rabbit, and will later be reported in more detail.[1]

It would appear therefore that radium injected intravenously or subcutaneously, either in the form of a bromide, or as an "active deposit" solution, will, after a short time, reach all parts of the animal body. Attention is called to the observation of Salant and Meyer, that radium after a subcutaneous injection is eliminated by way of the kidneys, the liver, and the small intestine. In such a case the lungs were not called upon to eliminate the radium, at least not to the extent recorded for the other organs. This observation would tend to explain the results regarding the lung changes noted in the animals of the present investigation.

In series B there was much less lung damage following a subcutaneous injection, than was recorded for the lungs in series A, where intravenous injections were given. It would appear from the work of Meyer and the present writer, that when radium is placed directly in the blood stream of an animal the lungs of that animal are exposed to a considerable amount of radioactivity.

From a careful histological study of the results of these experiments, no data have been obtained showing that the nucleoplasm of the cell is more severely damaged, or damaged more quickly, than the cytoplasm. To be sure, atypical nuclei have often been noted, but these were associated with severe degenerative changes in the cytoplasm as well. The cytoplasm in the degenerating cells was clumped in granules, and it appeared that water had been taken in by the cell to a considerable extent. This condition might very well have resulted from a change in the permeability of the cell membrane, due to the destructive

[1] Since the above was written the author has found that in 1914 Dr. William Duane reported a similar experiment before the American Philosophical Society. His results gave a somewhat higher proportion of deposited activity in the spleen and liver.

action of the radium rays. It seems probable that following such a condition the cell metabolism would be interfered with, and the nucleoplastic changes that subsequently occur would be but secondary in character, or at least simultaneous but not primary.

SUMMARY AND CONCLUSIONS

1. Following injections of an "active deposit" of radium emanation there is a diffusion of the radio-active substance throughout the animal body, which results in pathological changes in the various organs.

2. Pathological changes occurring in the liver, lungs, kidneys, adrenals, spleen, bone marrow, brain, and vascular system were presented in detail. The most interesting changes were those that were found in the liver, and resulted from comparatively small doses of radium injected subcutaneously. A fatty degeneration, associated with many giant cells and hyperchromatic nuclei, was found in the liver for a comparatively long time after the treatment.

3. Following large doses of radium, congestion and hemorrhages were frequently found in practically all the organs and in the severe, acute cases the animals died after showing symptoms of marked enteritis.

4. The most frequent pathological condition that occurred in the kidney was a granular degeneration and erosion of the renal cells.

5. It was found that injections of radium resulted in the destruction of the cells of the bone marrow, and replacement by blood.

6. Congestion of the spleen was the most constant feature following radium treatment, and in some cases this was associated with hemorrhages, and the destruction of red blood cells.

7. The method of injection appears to determine, to a certain extent, the severity of reaction in certain organs. For example, following subcutaneous injections there was comparatively no pathological reaction, of an appreciable extent, in the lungs, but with intravenous doses, of about the same strength,

the lung lesions were severe, and consisted of proliferation and desquamation of the epithelial cells of the bronchi, marked edema, congestion, and hemorrhage.

8. It appears that doses of radium less than 10 mc. are sublethal for the animals of this investigation. Doses above this amount may kill within a few hours to a few days after treatment, the reaction being somewhat less severe if the subcutaneous rather than the intravenous method of injection is used.

9. A similarity was noted in tissue reaction between radium injected intravenously or subcutaneously, and radium applied externally.

10. The fate of radium, after its injection in the animal organism and its subsequent elimination, was discussed in some detail. The results of this investigation tend to show that the liver, gastrointestinal tract, kidneys, lungs, and spleen receive the greatest amount of radio-activity.

11. Degenerative changes in the cell, and their possible interpretation were also discussed. The histological study tends to show that cytoplasmic changes occurring in the cell are profound, and as severe as the changes in the nucleoplasm.

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(10) Salant and Meyer: Am. Jour. Physiol., 1907–08, xx, 366.

PLATE 1

Kidney of animal VIII, series A. Death occurred two and a half days after the intravenous injection of 11.2 mc. of radium. Note the congestion and hemorrhage. (For further reference see text.)

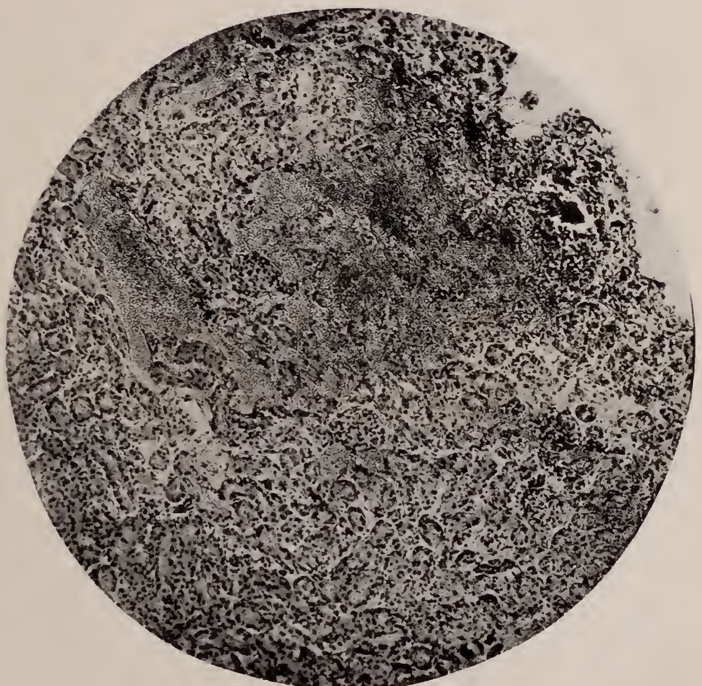

PLATE 2

Liver of animal X, series B. The animal died five days after a subcutaneous injection of 20.5 mc. of radium. The central area shows a region of fairly normal liver cells, on either side there is severe congestion and degeneration with the presence of giant cells. (For further reference see text.)

THE RELATION OF PREGNANCY AND REPRODUCTION TO TUMOR GROWTH[1]

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

PROBLEMS IN THE BEHAVIOR OF TUMORS

Tenth Report

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Every biological fact concerning the behavior of cancer is a step toward the solution of the problems of that disease. Biological data help to form the very foundation of the most advanced medical science today. This is particularly true in regard to neoplasms, which have in many of their aspects, including heredity, been shown to conform closely to laws which are an established part of modern biology and which are no longer under dispute.

These biological data, then, cannot be brushed aside because we do not wish to believe them[2] or ignored because they interfere with some preconceived idea.

The literature of the experimental laboratory gives little data on the influence of pregnancy upon the rate of tumor growth. Leo Loeb (2) in his paper on Tissue Growth and Tumor Growth discusses in detail the general factors modifying tumor growth. In this paper he states that "in the rat, pregnancy seems to favor

[1] Delivered before the American Society for Cancer Research, Atlantic City, June 14, 1919.

[2] Ewing, J.: Neoplastic Diseases, Philadelphia, 1919, p. 107: "Nothing authorizes us to affirm that cancer is hereditary. In the interests of the public this doctrine ought to be combated."

growth of embryonal tissues under certain conditions; in the mouse it is unfavorable to such growth." Later in this same paper, he states "it will be necessary to distinguish more sharply than has been done in the past between the effect of pregnancy on the growth of *spontaneous* and of *transplanted* tumors." Whether the growths referred to above were spontaneous or transplanted is not stated. Woglom (3), reviewing this phase of cancer, said "It has been asserted and denied that the existence of pregnancy rendered animals less susceptible to *implantation*." Haaland found that pregnancy often exerted an inhibitory influence upon the proliferation of tumors, the effect of which was to produce a striking retardation of their growth in pregnant animals as compared with animals not bearing young.

Clinical literature yields case reports from which diverse conclusions are drawn. Bainbridge (1), reporting two of his own cases, states: "from these and other personal observations, as well as from the literature, it is apparent that pregnancy exercises a stimulating and hence a malign influence on coexistent cancer in any part of the body."

Bainbridge reviews the literature, and for purposes of ready reference I have added his bibliography to this article. He states:

> Siebold maintains he has observed a spontaneous cure of genital cancer owing to a supervening pregnancy. Pinard considers the rapid growth of cancer during pregnancy by no means proved, and Varnier reports a somewhat remarkable case: "In October 1897 the presence of an enormous carcinoma of the portio was ascertained in a pregnant woman. The following year there was again a pregnancy and death did not take place until October 1900."

Ewing (5) says: "Pregnancy has an unfavorable influence on the course of many uterine carcinomas, but this influence is not always apparent."

There is, therefore, in the light of these conflicting statements, need of exact data from the experimental laboratory on this subject, which in its fundamentals is entirely a biological subject, but whose clinical importance is very great.

This paper presents exact biological data concerning the behavior of spontaneous tumors in their relation to pregnancy and reproduction. In all this work nature is allowed to take her own course and is not interfered with in any way. In order to follow the most rigid exactions, I have confined this study to tumors of a specific type and of a specific organ, viz: alveolar tubular carcinoma of the mammary gland, some portions of some of the tumors being cystic. For purposes of comparison, a few other types have been introduced.

Five years ago I reported briefly on this point (4) and the subject has been under study ever since. The data which are here given represent, therefore, a portion of the accumulated evidence of five years and are perfectly typical of the behavior of tumor growth in its relation to pregnancy and reproduction.

In handling large numbers of these mice with spontaneous tumors, there is forced upon the observer from the very first (1) the tremendous difference in the rate of tumor grown in the non-reproducing and in the reproducing females; (2) the same tremendous difference in the amounts of tumor grown by a reproductive female during her reproductive and her non-reproductive periods.

The point is of such importance in the etiology of neoplasms as to warrant a very complete analysis of the facts.

For this demonstration I have selected thirty females not reproducing after the appearance of their tumors, but all of which had previously borne young; and thirty females constantly reproducing after the appearance of their tumors, all of which also had previously borne young. The data concerning the latter are divided into two periods, (1) the reproductive and (2) the non-reproductive periods. Charts 1 to 4 concern the females not reproducing after the appearance of their tumors, and give the following information on each mouse:

1. The duration of the tumor from the date found to the date of death.
2. The number of tumors.
3. The type of tumor.
4. Size in millimeters of the tumor when found.

5. Size of the tumor at death.
6. Causes of death.
7. Age of the mouse in days.
8. Total amount of tumor growth in cubic millimeters.
9. Average daily rate of tumor growth in cubic millimeters.

FEMALES NOT REPRODUCING AFTER THE APPEARANCE OF THEIR TUMORS

1. Female 6357 was picked up December 26, 1913, with a tumor in the right axilla 12 x 8 x 8 mm. or 768 cmm. She lived twenty-two days, during which time the first tumor became 30 x 30 x 28

CHART 1

No	Duration of Tumor	No. of Tumors	Type	Size in MM when found	Size in MM at Death	Cause of Death	Age	Rate of Tumor Growth Cmm in Days	Average Daily Rate of Tumor Growth cmm
6357	Dec. 26 '13- Jan. 17 '14 22 Days	3	(1) Rt. Ax. M G. Solid Tub Carc. (2) Rt Fl. M G. ditto (1) (3) Lf Fl. M. G ditto (1) Lungs nearly replaced metas	(1) 12 x 8 x 8 768 cmm (2) ——— (3) ———	(1) 30 x 30 x 28 25200 cmm (2) 10 x 6 x 6 360 cmm (3) 16 x 12 x 10 1920 cmm	Tumors Gastric Hem	Dec 23 '12 Jan 17 '14 390 Days	(1) 24432 (2) 360 (3) 1920 26712 in 22	1214.18
6784	Feb 17' Mch. 8 '14 19 Days	3	(1) Lf. Ing. M. G Hem. Cyst Tub Carc. (2) Rt. Fl. M. G. Small-Cell- Tub. Carc. (3) Lf. Ax. M. G. Hem. Cyst Carc Tumor Emboli in Lungs	(1) 10 x 5 x 5 250 cmm (2) ——— (3) ———	(1) 30 x 25 x 20 15000 cmm (2) 18 x 6 x 6 648 cmm (3) 6 x 6 x 6 216 cmm	Pulmonary Infect.	Mch 14 '13 Mch. 8 '14 356 Days	(1) 14750 (2) 648 (3) 216 15614 in 19	821.78
6933	Feb. 15 '14 Mch. 24 '14 37 Days	4	(1) Rt. Ax. M G Alv Carc. (2) Rt Sub-Ax MG Hem. Ad. Carc (3) Lf Ant. M G ditto (2) (4) Rt Ing M G Cyst Ad Carc.	(1) (2) 4 x 2 x 2 16 cmm (3) ——— (4) ———	(1) 12 x 12 x 8 1152 cmm (2) 25 x 20 x 15 7500 cmm (3) 14 x 14 x 10 1960 cmm (4) 8 x 6 x 6 288 cmm	Tumors Chronic Nephritis	Apr. 26 '12 Mch. 24 '14 669 Days	(1) 1152 (2) 7484 (3) 1960 (4) 288 10884 in 37	294.16
7025	Mch. 1- Apr. 7 '14 37 Days	2	(1) Lf Ing. M G Hem. Carc (2) Rt Fl M G ditto (1) Mult. Metas Lungs	(1) 8 x 4 x 4 128 cmm (2) ———	(1) 40 x 30 x 30 36000 cmm (2) 8 x 6 x 6 288 cmm	Tumors Amyloidosis	Mch. 7 '13 Apr 7 '14 396 Days	(1) 35872 (2) 288 36160 in 37	977.29
5437	July 29 '13 Sept. 3 '13 36 Days	1	Ing M G. Solid Med. Carc.	5 x 3 x 3 45 cmm	45 x 30 x 25 33750 cmm	Tumors Hyp Heart Term Infec	Sept '14 12 Sept. 3 13 354 Days	33705 in 36	936.25
6415	Dec. 3 '13 Jan 26 '14 54 Days	3	(1) Rt. Fl. M G. Alv. Tub Carc (2) Lf Fl. M G Alv. Tub. Carc (3) Rt Ax. M G. ditto (2)	(1) 6 x 3 x 2 36 cmm (2) ——— (3) ———	(1) 30 x 26 x 20 15600 cmm (2) 3 x 3 x 3 27 cmm (3) 1.5 x 1.5 x 1.5 3.37 cmm	Tumors Gastric Hem	Sept 14 12 Jan 26 '14 488 Days	(1) 15564 (2) 27 (3) 3.37 15594 37 in 54	288.78
5581	Aug. 29- Sept. 24 '13 26 Days	2	(1) Rt. Ing. M. G Sq. cell Carc. (2) Lf. Lower Lung. Adenoma C.	(1) 10 x 6 x 6 360 cmm (2) ———	(1) 40 x 35 x 30 42,000 cmm (2) 2 x 2 x 2 8 cmm	Tumor Chronic Nephritis	Sept 28 '12 Sept 24 '13 361 Days	(1) 41640 (2) 8 41648 in 26	1601.84

mm. or 25,200 cmm. in size, while there also developed a second tumor of 360 cmm., and a third of 1920 cmm. She died January 17, 1914, of tumors and a gastric hemorrhage. She was then, at death, 1 year and 25 days, or 390 days old. During the period of tumor growth, 22 days, she grew 26,712 cmm. of tumor, an average of 1214.18 cmm. daily.

2. Female 6784, was found February 17, 1914, with a tumor 10 x 5 x 5 mm., or 250 cmm., in the left inguinal mammary gland. She died at 356 days of age, the original tumor having grown 14,750 cmm., while two other tumors of 648 cmm. and 216 cmm., respectively, had developed. She grew then 15,614 cmm. of tumor in 19 days, an average of 821.78 cmm. daily, dying of pulmonary infection.

3. Female 6933 died at 669 days old, of chronic nephritis, with a tumor growth of 10,884 cmm. in 37 days, an average of 294.16 cmm.

4. Female 7025 died at 396 days, of tumors and general amyloidosis, with a total tumor growth of 36,160 cmm. in 37 days, or an average growth of 977.29 cmm.

5. Female 5437 had a tumor of 45 cmm. when found; at death, 36 days later, the tumor measured 33,705 cmm., an average growth of 936.25 cmm. daily. She died when she was 354 days old of a terminal infection, with hypertrophied heart.

6. Female 6415, sister of 5437, died of tumors and a gastric hemorrhage at 488 days of age. She grew 15,594.37 cmm. of tumor in 54 days, an average of 288.78 cmm. daily.

7. Female 5581 was picked up August 29, 1913, with a right inguinal mammary gland tumor 10 x 6 x 6 mm. (360 cmm.). She died September 24, or 26 days later, of tumor and chronic nephritis, her tumor then measuring 42,000 cmm. She had grown 41,648 cmm. of tumor in 26 days, an average daily growth of 1601.84 cmm.

8. Female 5698 was found on September 15, 1913, with a tumor 8 x 5 x 4 mm. (160 cmm.) in the left inguinal mammary gland. She lived thirty days, during which time her first tumor grew 30,696 cmm. and she developed three other tumors of 2520, 125 and 240 cmm., respectively. She died at 352 days of age with terminal infection involving edema of the lungs and hydrothorax. She thus grew 33,581 cmm. of tumor in 30 days, an average of 1119.36 cmm.

9. Female 5723 on September 12, 1913, exhibited a left axillary mammary gland tumor 5 x 4 x 2 mm. or 40 cmm. She lived 36 days, growing 60,760 cmm. of tumor or an average of 1687.77 cmm.

At necropsy she disclosed a medullary carcinoma with multiple tumor emboli in the lungs.

10. Female 5753, sister of 5581, was found September 13, 1913, with a tumor of 240 cmm. in the right anterior mammary gland. She lived 27 days during which time her first tumor grew to be 45 x 45 x 30 mm. or 60,750 cmm., while a second tumor of 800 cmm. also developed. She grew then 61,310 cmm. of tumor in 27 days or an average of 2270.74 cmm. She died of her tumors at the age of 216 days.

CHART 2

No	Duration of Tumor	No. of Tumors	Type	Size in mm. when Found	Size in mm. at Death	Cause of Death	Age	Rate of Tumor Growth cmm in Days	Average Daily Rate of Tumor Growth - cmm.
5698	Sept. 15 - Oct. 15. 13 30 Days	4	(1) Lf. Ing. M.G. Cyst. Alv. Carc. (2) Lf. Fl. M.G ditto (1) (3) Rt. Fl. M.G ditto (1) (4) Ant. to (3) ditto (1) Pul. Metas.	(1) 8x5x4 160 cmm (2) —— (3) —— (4) ——	(1) 38 x 28 x 29 30856 cmm (2) 18 x 14 x 10 2520 cmm (3) 5 x 5 x 5 125 cmm (4) 10 x 6 x 4 240 cmm	Tumors Edema of Lungs. Hydrothorax Terminal Infection	Oct. 27. 12 Oct. 15. 13 352 Days	(1) 30696 (2) 2520 (3) 125 (4) 240 33581 in 30	1119.36
5723	Sept 12 - Oct. 18 13 36 Days	1	Lf. Ax. M.G. Med Carc Tumor Emboli Lungs	5x4x2 40 cmm	40 x 40 x 38 60800 cmm	Tumor	Dec. 7 '12 Oct. 18 '13 325 Days	60760 in 36	1687.77
5753	Sept 13 Oct. 20 '13 27 Days	2	(1) Rt. Ant. M.G Cyst. Hem. Alv. Carc (2) Rt Ax. M.G ditto (1)	(1) 8x6x5 240 cmm (2) ——	(1) 45x45x30 60750 cmm (2) 8 x 10 x 10 800 cmm.	Tumors	Mch. 18 '13 Oct. 20' 13 216 Days	(1) 60510 (2) 800 61310 in 27	2270.74
6001	Oct. 9 - Nov. 23 '13 45 Days	3	(1) Rt. Ax. M.G. Alv. Tub. Carc. (2) Sub-Ax. M.G Cyst. Hem. Carc (3) Lf. Ant. M.G. Alv. Carc. Lf. Lower Lung Tumor Emboli	(1) 6x3x3 54 cmm (2) —— (3) ——	(1) 22 x 20 x 10 4400 cmm (2) 15 x 13 x 10 1950 cmm (3) 4x4x4 64 cmm	Tumors Abscess in Liver. Gen. Infect.	June 2 '12 Nov. 23 '13 549 Days	(1) 4346 (2) 1950 (3) 64 6360 in 45	141.33
7247	Apr. 6 - May 4 14 28 Days	3	(1) Rt. Ax. M.G. Alv. Tub. Carc. (2) Rt. Ing. M.G ditto (1) (3) Rt. Fl. M.G. ditto (1)	(1) 4x2x2 16 cmm (2) —— (3) ——	(1) 30 x 28 x 20 16800 cmm (2) 12 x 12 x 10 1440 cmm (3) 6 x 6 x 3 108 cmm.	Hyp. Heart Tumors	Apr. 18 '13 May 4 '14 381 Days	(1) 16784 (2) 1440 (3) 108 18332 in 28	654.71
7324	Apr. 1 - May 14 14 43 Days	1	Lf. Ant. M.G. Sq. Cell Carc.	4x4x2 32 cmm	40 x 40 x 30 48000 cmm.	Tumor Term. Infect	Mch. 12 '13 May 14 '14 428 Days	47968 in 43	1115.53
7467	Apr 20 - May 29 '14 39 Days	2	(1) Rt. Ing. M.G. Cyst. Alv. Carc (2) Pelvic Alv. Carc. Rt. Lung Riddled Metas	(1) 8x4x4 128 cmm (2) ——	(1) 43 x 30 x 30 38700 cmm (2) 20 x 18 x 15 5400 cmm	Tumors Amyloidosis	July 16 '13 May 29 '14 317 Days	(1) 38572 (2) 5400 43972 in 39	1127.48
7532	May 15 June 8 '14 24 Days	1	Lf. Ing. M.G. Alv. Tub Carc.	6x3x2 36 cmm	30 x 25 x 25 18750 cmm.	Tumor Term. Infect.	Jan. 25 '13 June 8' 14 489 Days	18714 in 24	779.75

11. Female 6001 was picked up on October 9, 1913, with a tumor 6 x 3 x 3 mm. (54 cmm.) in the right axilla. She lived 45 days after this date, her first tumor growing to 4400 cmm., along with two other tumors of 1950 cmm. and 64 cmm. respectively. She died at 549 days of age, necropsy showing an abscess in the liver with generalized infection and tumor emboli in the lung, in addition to the mammary gland tumors. She grew 6360 cmm. of tumor in 45 days, an average of 141.33 cmm.

12. Female 7247 was found on April 6, 1914, with a right axillary mammary gland tumor 4 x 2 x 2 mm. (16 cmm.). She lived 28 days thereafter during which time her first tumor became 30 x 28 x 20 mm. in size (16,800 cmm.). She grew also in this period a second tumor of 1440 cmm. and a third of 108 cmm. She died at 381 days of age, having grown 18,332 cmm. of tumor in 28 days, or an average of 654.71 cmm. daily.

CHART 3.

No.	Duration of Tumor	No. of Tumors	Type	Size in mm when found	Size in mm at death	Cause of Death	Age	Rate of Tumor Growth cmm in Days	Average Daily Rate of Tumor Growth cmm
7572	May 4 - June 16 '14 43 Days	6	(1) Lf. Fl. M.G. Tub. Carc. (2) Lf. Ax. M.G. Cyst. Tub. Carc. (3) Lf. Sub-Ax. M.G. Tub. Carc. (4) Adjacent to (3) Hem. Cyst. Carc. (5) Rt. Ant. M.G. Lymphoma (6) Lf. Ant. M.G. ditto (5)	(1) 4 x 4 x 3 48 cmm (2) 4 x 2 x 2 16 cmm (3) —— (4) —— (5) 4 x 4 x 4 64 cmm (6) ——	(1) 14 x 12 x 8 1344 cmm (2) 14 x 12 x 10 1680 cmm (3) 10 x 10 x 8 800 cmm (4) 5 x 5 x 5 125 cmm (5) 22 x 20 x 16 7040 cmm (6) 10 x 8 x 8 640 cmm	Tumors	Dec 5 '12 June 16 '14 558 Days	(1) 1296 (2) 1664 (3) 800 (4) 125 (5) 6976 (6) 640 11501 in 43	267.46
7761	May 25 July 8 '14 44 Days	1	Lf. Fl. M.G. Alv. Tub Carc Lungs riddled Metas	5 x 4 x 4 80 cmm	45 x 40 x 35 63000 cmm	Atrophied Organs from Tumor Pressure	July 19 '13 July 8 '14 354 Days	62920 in 44	1430.0
5357	July 15 - Aug. 25 '13 41 Days	1	Rt. Ant. M.G. Tub. Carc	4 x 4 x 4 64 cmm	40 x 30 x 25 30000 cmm	Hyp. Heart Tumor	July 6 '12 Aug 25 '13 416 Days	29936 in 41	730.14
8042	July 5 - Aug. 13 '14 39 Days	2	(1) Rt. Ax. M.G. Alv. Tub. Carc (2) Lf. Ing. M.G. ditto (1) Lungs Metas	(1) 5 x 3 x 3 45 cmm (2) ——	(1) 35 x 28 x 25 24500 cmm (2) 6 x 6 x 6 216 cmm	Tumors	June 30 '13 Aug. 13 '14 409 Days	(1) 24455 (2) 216 24671 in 39	632.58
8104	July 1 - Aug. 21 '14 51 Days	3	(1) Lf. Fl. M.G. Sq. cell Carc. Metas Reg. Gland (2) Rt. Ing. M.G. Alv Tub Carc (3) Lf Ing M G Sq. cell Carc. Lungs replaced Metas	(1) 6 x 5 x 3 90 cmm (2) —— (3) ——	(1) 45 x 40 x 35 63000 cmm. (2) 2 x 2 x 2 8 cmm (3) 1.5 x 2 x 2 6 cmm	Tumors Terminal Infect	Oct 2 '13 Aug 21 '14 323 Days	(1) 62910 (2) 8 (3) 6 62924 in 51	1233.80
8573	Sept 15 - Oct 23 '14 38 Days	3	(1) Rt. Fl. M.G. Alv. Tub Carc (2) Lf Ant. M.G. ditto (1) (3) Lf. Ax. M.G ditto (1)	(1) 5 x 4 x 2 40 cmm (2) —— (3) ——	(1) 40 x 30 x 30 36000 cmm (2) 3 x 3 x 3 27 cmm (3) 2 x 2 x 2 8 cmm	Tumors	Oct. 28 '13 Oct. 23 '14 359 Days	(1) 35960 (2) 27 (3) 8 35995 in 38	947.23
8911	Oct. 29 - Dec 6 '14 38 Days	1	Rt. Ax. M.G. Alv. Ad. Carc Lungs Mult. Emboli	8 x 5 x 3 120 cmm	45 x 40 x 35 63000 cmm	Tumors	Apr. 23 '14 Dec 6 '14 227 Days	62880 in 38	1654.73

A study of the charts will show parallel data concerning each of the other eighteen individuals in the class of mice which did not reproduce after the appearance of a tumor; in order to conserve space the other case reports are given in the charts only. The average daily rate of growth for the total number of these mice was 999.45 cmm. The youngest of these mice was 216 days, the oldest 803 days. The average age then, was 415 days or 1 year, 1 month, 21 days.

The second set of charts, nos. 5 to 8, deals with the reproducing females and gives the same items as the first set with the addition of the following:

10. The number of litters born after the appearance of tumor.
11. The total number of young after the appearance of tumor.
12. Time between the date of the last litter and the death date.

The total tumor growth and the average daily rate of growth are given in two groups: (1) the reproductive period, (2) the non-reproductive period.

CHART 4

No	Duration of Tumor	No. of Tumors	Type	Size in MM when Found	Size in MM at Death	Cause of Death	Age	Rate of Tumor Growth CMM. in Days	Average Daily Rate of Tumor Growth CMM
9365	Dec. 2 '14 Jan. 6 '15 35 Days	2	(1) Rt. Ing. M.G. Sq-cell. Carc. Lung Metas. (2) Pelvic Sq-cell Carc.	(1) 10 x 10 x 6 600 cmm (2) ——	(1) 35 x 35 x 40 49000 cmm (2) 10 x 10 x 10 1000 cmm	Tumors Edema. Lungs	Mch. 1 '14 Jan. 6 '15 311 Days	(1) 48400 (2) 1000 49400 in 35	1141.42
9510	Jan. 5 - Feb. 24 '15 50 Days	1	Lf. Ax. M.G Alv. Carc. Lung Metas.	6 x 2 x 2 24 cmm	45 x 40 x 40 72000 cmm	Tumor Chronic Nephritis	May 1 '14 Feb. 24 '15 299 Days	71976 in 50	1439.52
1053	Feb. 21 - Mch. 14 '12 22 Days	2	(1) Ing. M.G Sol. Med. Carc (2) Rt. Ant. M.G Cyst. Carc.	(1) 5 x 4 x 2 40 cmm (2) ——	(1) 35 x 22 x 17 13090 cmm (2) 8 x 5 x 5 200 cmm	Tumors Amyloidosis	Mch. 8 '11 Mch. 14 '12 371 Days	(1) 13050 (2) 200 13250 in 22	602.27
10244	Apr. 18 May 18 '15 30 Days	1	Lf. Ax. M.G. Alv. Tub. Carc.	10 x 5 x 4 200 cmm	40 x 35 x 35 49000 cmm	Tumor Inflam. Lungs	Sept 7 '14 May 18 '15 253 Days	48800 in 30	1626.66
10253	Apr. 1 - May 19 '15 48 Days	4	(1) Lf. Ant. M.G. Alv. Tub. Carc (2) Rt. Ant. M.G ditto (1) (3) Rt. Fl. M.G. ditto (1) (4) Rt. Lower Lung Pap.	(1) 3 x 3 x 2 18 cmm (2) —— (3) —— (4) ——	(1) 14 x 10 x 10 1400 cmm. (2) 14 x 10 x 10 1400 cmm (3) 14 x 10 x 10 1400 cmm (4) 2 x 2 x 2 8 cmm.	Infected Tumors Terminal Infect	Mch. 7 '13 May 19 '15 803 Days	(1) 1382 (2) 1400 (3) 1400 (4) 8 4190 in 48	87.29
10335	Apr. 21 - May 29 '15 38 Days	2	(1) Ant. Dorsal Mid-line M.G Tub. Carc (2) Rt. Ax. M.G ditto (1)	(1) 8 x 8 x 8 512 cmm (2) ——	(1) 45 x 35 x 35 55125 cmm (2) 3 x 3 x 3 27 cmm	Tumors Terminal Infect.	Aug. 28 '14 May 29 '15 272 Days	(1) 54613 (2) 27 54640 in 38	1437.89
19054	Jan. 17 - Feb. 28 '18 42 Days	3	(1) Rt. Ax. M.G. Alv. Tub. Carc. (2) Rt. Ant. M.G ditto (1) (3) Lf. Fl. M.G ditto (1)	(1) 8 x 4 x 4 128 cmm (2) 4 x 2 x 2 16 cmm (3) ——	(1) 40 x 35 x 30 42000 cmm (2) 20 x 16 x 16 5120 cmm (3) 8 x 6 x 6 288 cmm	Tumors	Feb. 1 '17 Feb. 28 '18 392 Days	(1) 41872 (2) 5104 (3) 288 47264 in 42	1125.33
13565	June 1 - July 2 '16 31 Days	4	(1) Lf. Ax. M.G Pap. Carc. (2) Lf. Ing. M.G. Pap. Ad. Carc (3) Rt. Ax. M.G. ditto (2) (4) Lf. Lower Lung Carc Lung Metas	(1) 8 x 4 x 4 128 cmm (2) —— (3) —— (4) ——	(1) 36 x 25 x 20 18000 cmm (2) 8 x 6 x 6 288 cmm (3) 3 x 2 x 2 12 cmm (4) 8 x 6 x 6 288 cmm	Tumors	Jan. 4 '15 July 2 '16 544 Days	(1) 17872 (2) 288 (3) 12 (4) 288 18460 in 31	595.48

FEMALES CONSTANTLY REPRODUCING AFTER THE APPEARANCE OF TUMORS

1. Female 2426 was found June 16, 1912, with a tumor 10 x 5 x 5 mm. (250 cmm.) in the left inguinal mammary gland. She lived until September 7, 1912, or 83 days after the appearance of her tumor, during which time she produced 4 litters, with a total of 19 young. The last litter was born dead the day

CHART 5.

No.	Duration of Tumor	No. of Litters	No. of Young	No. of Tumors	Type.	Size in MM. When Found	Size in MM., At Death.	Cause of Death.	Time — Last Litter To Death	Age	Rate of Tumor Growth CMM in Days	Average Daily Rate of Tumor Growth CMM.
2426	June 16 — Sept. 7 '12 83 Days	4	19	1	Lf. Ing Tub. Carc. M.G.	10x5x5 250 Cmm.	15x15x15 3375 Cmm.	Hyp. Heart Thromb. Aur. Hydrothorax.	Sept. 6. (dead) Sept. 7 '12	June 27 '11 Sept. 7 '12 437 Days	3125 in 83	37.65 Reprod
3621	July 21 '12 Jan. 16 '13 179 Days	7	25	1	Rt. Ax. Alv. Tub Carc. M G	3x3x2 18 cmm Dec. 25 '12 8x3x2 48 cmm	16x8x8 1024 Cmm	Inf. with Liver Necrosis.	Dec. 25. '12 (dead) Jan. 16 '13	Dec. 5 '11 Jan. 16 '13 407 Days	30 in 157 976 in 22	.19 Reprod. 44.36 Non-Reprod.
4339	Sept. 15 '12 April 13 '13 210 Days	7	20	3	(1) Fl. to Fl Sm. cell Carc. M.G. (2) Rt. ant. Carc. M.G. (med-metas) (3) Lf. Lower Lung Pap Mult. Lung Metas	4x3x2 24 cmm Feb. 21 10x8x8 640 cmm	(1) 45x45x30 60750 cmm. (2) 2x2x2 8 cmm. (3) 4x4x4 64 cmm.	Tumors	Feb. 21 (dead) April 13 '13	Sept. 10 '11 April 13 '13 580 Days.	(1) 616 in 159 60110 in 51 (2) 8 in 51 (3) 64 in 51 60182 in 51	3.87 Reprod. 1182. Non-Reprod.
4554	Jan. 30 — May 15 '13 105 Days	4	15	1	Rt. Ax Tub Carc M G	6x4x2 48 cmm April 30 6x6x6 216 cmm.	10x10x10 1000 cmm	Gen. Sepsis from Infected Uterus with Dead Foetus	April 30 (dead) - May 15 '13	Aug. 6 '12 May 15 '13 282 Days	168 in 90 784 in 15	1.86 Reprod 52.26 Non-Reprod
4751	Feb 13 — June 9 '13 116 Days	4	25	1	Rt Ax Alv Tub Carc M.G Mult. Small Metas. Lungs	10x4x3 120 cmm May 9 '13 10x8x8 640 cmm.	42x32x18 24192 cmm	Tumor	May 9. — June 9. '13	July 3 '12 June 9 '13 341 Days	520 in 85 23552 in 31	6.11 Reprod 759.74 Non-Reprod
5417	Oct 24 '12 Sept. 1 '13 311 Days	7	23	3	(1) Ing. M.G. Lar. Cell Alv. Carc (2) Pelvic Lar. Cell Carc (3) Rt. Fl. M.G Tub. Cyst. Carc. Mult. Lung Metas from (3)	(1) 8x5x5 200 cmm July 1 '13 10x8x6 480 cmm	(1) 52x42x28 61152 cmm. (2) 14x14x14 2744 cmm (3) 4x4x4 64 cmm	Tumors	July 1 (dead) Sept. 1. '13	April 30 '12 Sept. 1 '13 488 Days	(1) 280 in 250 60672 in 61 (2) 2744 in 61 (3) 64 in 61 63480 in 61	(1) 1.12 Reprod 994.62 Non- " (2) 44.98 " " (3) 1.04 " " 1040.64 Non-Rep.
5673	Dec. 31 '12 - Oct. 11 '13 284 Days	6	24	2	(1) Rt Ax. M.G. Tub. Cyst. Carc (2) Lf Ing. M.G Tub Carc	(1) 8x5x5 200 cmm June 5 10x8x8 640 cmm	(1) 35x30x18 18900 cmm. (2) 10x10x10 1000 cmm	Chr Neph	June 5 - Oct. 11 '13	Dec. 16 '10 - Oct. 11 '13 1024 Days	(1) 440 in 156 17360 in 128 (2) 1000 in 128 18360 in 128	(1) 2.82 Reprod 135.62 Non-Rep. (2) 7.81 " " 143 43 Non. Rep
6981	Oct. 1 '13 Mch. 31 '14 181 Days	6	30	1	Lf. Fl. M.G. Alv Carc	6x5x4 120 cmm Feb. 20 '14 10x8x8 640 cmm	25x15x15 5625 cmm	Infected Tumor. Gen. Sepsis - Amyloidosis	Feb. 20 '14 (dead) Mch. 31 '14	Jan. 30 '13 March 31 '14 425 Days	520 in 142 4985 in 39	2.95 Reprod 127.80 Non-Rep.
7246	Dec. 19 '13 May 5 '14 137 Days	5	21	1	Rt to Lf Ax. M G Alv Tub Carc Mult Lung Metas Leukemia	8x5x5 200 cmm. Apr. 4 '14 8x8x8 512 cmm	34x34x30 34680 cmm	Tumor and Leukemia	Apr. 4 — May 5 '14	June 2 '13 May 5 '14 337 Days	312 in 106 34168 in 31	2.94 Reprod 1102.19 Non-Rep.

before her death, so that she had no non-reproductive period after the appearance of her tumor. She died at 437 days of age, of a hypertrophied heart with thrombosis of the left auricle and hydrothorax. She grew 3125 cmm. of tumor in 83 days, an average of 37.65 cmm. daily during her reproductive period.

2. Female 3621 was found July 21, 1912 with a right axillary mammary gland tumor 3 x 3 x 2 mm. (18 cmm.). She lived 179 days after the appearance of her tumor. She produced 25 young in 7 litters during this period. The date of her last litter, born dead, was December 25, 1912, after which she lived 22 days. She had then 179 days of tumor growth during 157 of which she was reproducing; during the remaining 22 days she had no young. During her reproductive period she grew 30 cmm. of tumor in 157 days, an average daily growth of 0.19 cmm. During her non-reproductive period of 22 days she grew 976 cmm. of tumor, an average daily growth of 44.36 cmm.

3. Female 4339 was found September 15, 1912, with a tumor 4 x 3 x 2 mm. (24 cmm.) in the right flank mammary gland. She produced 20 young in 7 litters while growing her tumor. The last litter was born dead February 21, 1913. She lived 210 days after the appearance of her tumor during which time her first tumor grew to 45 x 45 x 30 mm. (60,750 cmm.) and a second and third tumor of 8 cmm. and 64 cmm. respectively developed. She died at the age of 580 days, having grown 616 cmm. of tumor in the 159 days she was reproductive, or an average of 3.87 cmm. daily. During her non-reproductive period of 51 days, she grew 60,182 cmm. of tumor, or a daily average of 1182 cmm.

4. Female 4554 was found January 30, 1913, with a tumor 6 x 4 x 2 mm. (48 cmm.), after which she produced 4 litters with a total of 15 young. Her last litter was delivered dead, on April 30, at which time her tumor measured 6 x 6 x 6 mm. (216 cmm.). She died May 15, at 282 days of age, of general sepsis from infected uterus with a dead undelivered fetus. At her death her tumor measured 10 x 10 x 10 mm. or 1000 cmm. She grew then during her reproductive period 1.86 cmm. daily; while during her non-reproductive period she grew 52.26 cmm. daily.

5. Female 4751 on February 13, 1913, showed a right axillary mammary gland tumor 10 x 4 x 3 mm. (120 cmm.). Between that date and May 9, she had 4 litters, a total of 25 young. On the date of her last litter her tumor measured 10 x 8 x 8 mm. (640 cmm.). Between May 9 and June 9, her tumor grew to 42 x 32 x 18 mm. (24,192 cmm.). She showed at necropsy multiple lung metastases. During her reproductive period, 85 days, she grew 520 cmm., an average of 6.11 cmm. daily. During her non-reproductive period, 31 days, she grew 23,552 cmm. of tumor, 759.74 cmm. average daily growth.

6. Female 5417 was found October 24, 1912, with an inguinal mammary gland tumor 8 x 5 x 5 mm. (200 cmm.). She bore 7 litters after this date, a total of 23 young, the last a litter of 1 born dead July 1, 1913. On this latter date her tumor was still only 10 x 8 x 6 mm. (480 cmm.). She lived until September 1, 1913, her tumor at death being 52 x 42 x 28 mm. (61,152 cmm.). She had also grown a pelvic tumor 14 x 14 x 14 mm. (2744 cmm.) and a right flank mammary gland tumor of 64 cmm. At necropsy her lungs showed multiple small metastases. During her reproductive period then, 250 days, she grew only 280 cmm. of tumor, an average of 1.12 cmm. daily. During her non-reproductive period of 61 days she grew 63,480 cmm., an average of 1040.64 cmm. daily.

7. Female 5673 was picked up December 31, 1912, with a right axillary mammary gland tumor 8 x 5 x 5 mm. (200 cmm.). She bore 6 litters, a total of 24 young, after this date. Her last litter was born June 5, 1913, at which date her tumor measured 10 x 8 x 8 mm. (640 cmm.). She died October 11, 1913, of chronic nephritis, being 1024 days old (2 years, 9 months, 24 days). She showed at necropsy a second tumor 10 x 10 x 10 mm. (1000 cmm.). During her reproductive period, 156 days, she grew 440 cmm. of tumor or an average of 2.82 cmm. daily, while during her non-reproductive period, 128 days, she grew 18,360 cmm. or 143.43 cmm. daily.

8. Female 7246 was found December 19, 1913, with a right axillary mammary gland tumor 8 x 5 x 5 mm. (200 cmm.). She bore 5 litters totalling 21 young after this date. Her last

CHART 6

No	Duration of Tumor	No. of Litters	No. of Young	No. of Tumors	Type	Size in mm when Found.	Size in mm at Death	Cause of Death	Time-Last Litter to Death	Age	Rate of Tumor Growth Cmm in Days	Average Daily Rate of Tumor Growth ·Cmm
7454	Sept 26 13 May 26 14 242 Days	6	20	1	Rt to Lf Ax. M.G. Sp Cell Sarcoma	10x10x10 1000 cmm. May 1'14 12x12x12 1728 cmm	42x40x30 50400 cmm	Tumor	May 1 - May 26 14	Feb. 21'13 May 26'14 459 Days	728 in 217 48672 in 25	3.35 Reprod 1946.88 Non-Rep
7536	Feb 20 June 9 14 109 Days	5	19	1	Lf Ax. M.G Par Ad. Carc Lungs Riddled Metas	6x6x6 216 cmm May 22'14 8x6x6 288 cmm.	14x12x10 1680 cmm	Nematodes Tapeworm Typ. Changes	May 22 June 9' 14	June 23'13 June 9 '14 351 Days	72 in 91 1392 in 18	.79 Reprod 77.33 Non-Rep
7555	Jan. 13'14 June 13'14 151 Days	4	18	3	(1) Rt. Ant M.G. Alv Tub Carc (2) Rt Ax M.G Scirr. Carc (3) Lf. Ax. M.G Scirr. Carc Mult Lung Metas.	(1) 8x8x8 512 cmm May 7'14 10x8x10 800 cmm	(1) 18x18x14 4536 cmm (2) 10x10x10 1000 cmm (3) 5x5x5 125 cmm	Hyp. Heart. Lung Tumors	May 7 – June 13'14	Jan. 19 13 June 13'14 510 Days	(1) 288 in 114 3736 in 37 (2) 1000 in - (3) 125 - - 4861 in 37	(1) 2.52 Reprod 100.97 Non-Rep. (2) 27 02 - (3) 3.37 - 131 36 Non Rep
8304	Mch. 14 Sept. 15 '14 185 Days	4	17	1	Rt Ant M G Cyl cell Carc	4x4x4 64 cmm June 1'14 5x5x5 125 cmm	10x10x10 1000 cmm	Hyp. Heart Hydro- thorax.	June 1 - Sept. 15 '14	Sept. 5 '12 Sept 15'14 740 Days	61 in 79 875 - 106	.77 Reprod 8.25 Non-Rep
8889	July 19 Dec 3'14 137 Days	5	20	4	(1) Lf to Rt. Ax. M. G 3 Nods Alv. Tub. pap. Carc Sp. cell Sarc Tub Carc (2) Lf. Fl. M.G Early Cyst. Carc Lungs Riddled Metas	10x10x10 1000 cmm Nov. 19 '14 20x20x20 8000 cmm	(1) 50x30x30 45000 cmm (2) 2x2x2 8 cmm	Tumors	Nov. 19. – Dec 3'14	Dec 7 '13 Dec 3'14 392 Days	(1) 7000 in 123 37000 - 14 (2) 8 - - 37008 - 14	(1) 56.91 Reprod 2462 85 Non " (2) .57 " " 2643.42 Non-R.
9053	Sept 2 - Dec. 27'14 116 Days	4	18	1	Lf Ax M.G Sol Alv Carc.	6x6x6 216 cmm Dec 21'14 7x6x6 252 cmm	10x10x8 800 cmm	Tape- worm Chr. Neph.	Dec 21. – Dec. 27'14	Mch. 9 '14 Dec 27'14 293 Days	36. in 110 548. in 6	32 Reprod 91.33 Non-R.
9097	Nov. 5 '14 Jan. 2 15 58 Days	3	10	1	Rt Ing. M.G. Alv Tub. Carc	10x10x10 1000 cmm Dec 20 '14 10x10x10 1000 cmm	14x12x10 1680 cmm	Hem Left Ovary	Dec. 20'14 Jan 2 '15	Mch. 12 '13 Jan. 2 '15 661 Days	0. in 45 680. in 13	0. Reprod 52 30 Non-R

litter was born April 4, 1914, at which date her tumor measured only 8 x 8 x 8 mm. (512 cmm.). She died May 5, 1914, of tumors and leukemia, when she was 337 days old. She grew during her reproductive period, 106 days, 2.94 cmm. of tumor daily. During her non-reproductive period, 31 days, she grew 34,168 cmm. or 1102.19 cmm. daily.

9. The most striking illustration in this set of females is number 9172. She was picked up August 30, 1914, with a tumor 10 x 10 x 10 mm. (1000 cmm.) in the right axilla. After this date she bore 4 litters totalling 15 young. The date of her last litter was December 1, 1914, when her tumor measured only 11 x 11 x 10 mm. (1210 cmm.), having grown but 210 cmm. in 93 days or 2.25 cmm. daily. She lived 40 days longer and died January 10, 1915, of tumors, aged 337 days. During her non-reproductive period her tumor grew 98,790 cmm., while a left axillary carcinoma also developed to the size of 12 x 6 x 6 mm. (432 cmm.) making a total tumor growth of 99,222 cmm. in 40 days or 2480.55 cmm. daily (Chart 8).

10. Female 9097 is an interesting case. She was an old mouse, 661 days old when she died. She was found November 5, 1914, with a right inguinal mammary gland tumor 10 x 10 x 10 mm. (1000 cmm.). She bore 3 litters, 10 young, after this date. Her last litter was born December 20, 1914, at which date her tumor measured the same as when found, 45 days earlier. She lived 13 days longer, dying January 2, 1915, her tumor then being 14 x 12 x 10 mm. (1680 cmm.). She grew then 680 cmm. of tumor in 13 days while she was non-reproductive, an average of 52.3 cmm. daily. Necropsy showed a blood clot in the left ovary (Chart 8).

Charts 7 and 8 which cover the rest of the thirty reproducing females, show the same marked increase in tumor growth during the non-reproductive period. The average daily tumor growth for this set of mice was as follows:

1. During the reproductive period 7.75 cmm. daily.
2. During the non-reproductive period 686.34 cmm. daily, or over 88 times as much daily tumor growth as during the reproductive period.

CHART 7.

No.	Duration of Tumor	No. of Litters	No. of Young	No. of Tumors	Type	Size in MM when found	Size in MM at Death	Cause of Death	Time-Last Litter to Death.	Age	Rate of Tumor Growth Cmm. in Days	Average Daily Rate of Tumor Growth—
10015	Nov 21 '14 Apr. 3 '15 133 Days	5	15	2	(1) Rt. Ing. M.G. Alv. Sarcoma Metas Lymph Gland (2) Rt. Ax. M.G. Partly Cyst. Sq. Partly Pap. Ad. Carc.	(1) 10x10x10 1000 cmm Mch. 21 '15 12x10x10 1200 cmm (2) ———	(1) 20x20x18 7200 cmm (2) 10x10x2 200 cmm	Liver Abscess Amyloidosis	Mch. 21- Apr. 3 '15	Mch. 11 '14 Apr. 3 '15 388 Days	(1) 200 in 120 6000 in 13 (2) 200 " " 6200 in 13	(1) 1.66 Reprod 461.53 Non-Rep (2) 15.38 " " 476.91 Non-Rep.
12189	Apr. 1 '15 Jan. 21 '16 295 Days	6	26	2	(1) Rt. Fl. M.G. Alv. Tub. Cyst. Carc. (2) Lf Fl. M.G Ditto (1)	(1) 8x8x8 512 cmm Dec 10 '15 10x10x10 1000 cmm (2) ———	(1) 25x20x20 10000 cmm (2) 4x4x4 64 cmm	Chr. Neph	Dec. 10 '15 Jan. 21 '16	Apr. 26 '14 Jan. 21 '16 655 Days	(1) 488 in 253 9000 " 42 (2) 64 " " 9064 in 42	(1) 1.92 Reprod 214.28 Non-R. (2) 1.52 " " 215.80 Non-Rep
12852	Aug. 18 '15 Apr 18 16 244 Days	8	31	3	(1) Lf. Ax. M.G. Sol. Alv. Carc (2) Lf. Fl. M.G Cyst. Hem. Carc (3) Rt. Mid. M.G Ditto (2)	(1) 8x8x8 512 cmm Mch. 1 '16 12x10x8 960 cmm (2) ——— (3) ———	(1) 30x25x25 18750 cmm (2) 20x18x18 6480 cmm (3) 10x6x6 360 cmm	Tumors	Mch. 1 '16 (Dead) Apr. 18 '16	Jan. 30 '15 Apr. 18 '16 443 Days	(1) 448 in 196 17790. " 48 (2) 6480 " " (3) 360 " " 24630 in 48	(1) 2.28 Reprod. 370.62 Non-Rep. (2) 135. " " (3) 7.5 " " 513.12 Non-Rep.
12963	Aug 3 15 May 3 16 274 Days	7	27	2	(1) Lf. Ant. M.G. to Dor. Mid-line Alv Carc (2) Rt Mid-Lung Pap Carc	(1) 8x8x8 512 cmm Apr 1 16 12x10x10 1200 cmm (2) ———	(1) 20x18x18 6480 cmm (2) 8x6x6 288 cmm	Hem. Ovary Lung Tumor	Apr 1 May 3 '16	Jan. 4 15 May 3 '16 485 Days	(1) 678 in 242 5280 " 32 (2) 288 " " 5568 " "	(1) 2.80 Reprod 161.87 Non-R (2) 9. " " 170 87 Non-R
15650	Mch 15 16 " 4 17 354 days	6	22	2	(1) Rt. Ing. M.G. Hem. Alv. Tub. Carc (2) Rt. Ant. M.G Ditto (1)	(1) 4x4x4 64 cmm Feb. 5 17 10x8x5 400 cmm (2) ———	(1) 20x20x20 8000 cmm (2) 8x8x8 512 cmm	Intest. Infect.	Feb. 5 - Mch 4 17	Aug 12 '15 Mch. 4 '17 569 Days	(1) 334 in 327 7600 " 27 (2) 512 " " 8112 in 27	(1) 1.02 Reprod 281 48 Non-Rep. (2) 18.96 299.44 Non-Rep.
16046	Nov 16 16 Apr 18 17 153 Days	5	17	2	(1) Rt. Ing. M.G Tub. Alv Carc (2) Rt Sub-ax. M.G (Ditto (1))	(1) 4x4x4 64 cmm Mch 11 '17 8x8x8 512 cmm (2) Mch. 27 '17 10x10x10 1000 cmm	(1) 18x16x16 4608 cmm (2) 11x11x11 1331 cmm	Chr. Neph.	Mch. 27- Apr 18 '17	Nov. 25 '15 Apr. 18 '17 509 Days	(1) 448 in 131 (2) 1000 in 131 1448 in 131 (1) 4096 in 22 (2) 331 " " 4427 in 22	(1) 3.41 Reprod (2) 7.63 " 11.04 Reprod (1) 186.18 Non-Rep (2) 15.04 " " 201.22 Non-Rep.
15990	Dec. 13 '16 Apr. 12 '17 120 Days	3	13	1	Lf Ax. M.G Hem Cyst Carc.	8x8x8 512 cmm Feb 28 '17 12x12x12 1728 cmm	40x40x40 64000 cmm	Tumor Pul. Inf with Hem.	Feb. 28 (dead) Apr. 12 '17	Apr 30 '16 Apr 12 '17 348 Days	1216 in 77 62272 in 43	15.79 Reprod 1448.18 Non-Rep

CHART 8

No.	Duration of Tumor	No. of Litters	No. of Young	No. of Tumors	Type	Size in MM When Found.	Size in MM at Death	Cause of Death	Time - Last Litter to Death	Age	Rate of Tumor Growth - Cmm in Days	Average Daily Rate of Tumor Growth Cmm
16371	Dec. 18 '16 May 22 '17 155 days	3	7	2	(1) Rt. Ax. M.G Alv Tub. Carc (2) Lft. Ax. M.G. Ditto (1)	(1) 8x8x8 512 Cmm Feb. 28 10x10x10 1000 Cmm (2) ———	(1) 35x35x30 36750 Cmm (2) 10x10x10 1000 Cmm	Hydrothorax Inf. Areas Lung	Feb 28 '17 May 22 '17	May 1 '16 May 22 '17 387 Days	(1) 488 in 72 35750 in 83 (2) 1000 in 83 36750 in 83	(1) 6.77 Reprod 430.72 Non-Rep (2) 12.04 " " 442.76 Non-Rep.
18845	Feb. 19 '17 Feb. 1 '18 347 days	6	32	2	(1) Rt. Ing. M.G. Alv Carc (2) Rt. Ant. M.G. to Spine Ditto (1) Every Lobe Lung Nearly Replaced - Metas	(1) 4x4x4 64 Cmm Dec. 9 '17 8x8x8 512 Cmm (2) Nov 25 '17 6x6x6 216 Cmm	(1) 25x20x18 9000 Cmm (2) 25x25x25 15625 Cmm	Tumors Acute Neph	Nov. 25 '17 Feb. 1 '18	July 5 '16 Feb 1 '18 576 Days	(1) 448 in 279 (2) 216 in 279 664 in 279 (1) 8488 in 68 15409 in 68 23897 in 68	(1) 1.60 Reprod (2) .77 " 2.37 Reprod (1) 124.82 Non-Rep. (2) 226.60 " " 351.42 Non-Rep.
17466	May 16 '17 Aug. 30 '17 106 days	5	13	1	Rt. Ax. M.G Alv Tub. Carc.	4x4x4 64 Cmm Aug 14 '17 8x8x8 512 Cmm	18x18x18 5832 Cmm	Chr Neph.	Aug. 14 - Aug 30 '17	Oct. 1 '16 Aug. 30 '17 333 Days	448 in 90 5320 in 16	4.97 Reprod. 332.50 Non-Rep.
15768	(1) Oct. 23 '16 Mch. 19 '17 (2) Feb. 27 '17 Mch. 19 '17 (3) T. Eye Sept. 29 '16 Mch. 19 '17 147 days	4	18	3	(1) Lft. Ing. M.G. Cyst-Alv. Tub. - Carc. (2) Lft Sub. Ax. M.G. Alv. Tub. Carc. (3) Lft. Lower Lung Nearly Replaced - Pap	(1) 8x8x8 512 Cmm Feb. 27 '17 12x12x12 1728 Cmm (2) 10x10x10 1000 Cmm (3) ———	(1) 25x25x25 15625 Cmm (2) 25x20x20 10000 Cmm (3) 10x10x10 1000 Cmm	Tumors Chr Neph	Feb. 27 (dead) March 19 '17	Apr. 1 '16 Mch 19 '17 352 Days	(1) 1216 in 127 13897 in 20 (2) 9000 in 20 (3) 1000 in 20 23897 in 20	(1) 9.57 Reprod 694.85 Non-Rep. (2) 450 00 " " (3) 50.00 " " 1194.85 Non-Rep.
9172	Aug. 30 '14 Jan. 10 '15 133 days	4	15	2	(1) Rt Ax. M.G Alv. Carc. (2) Lf. Ax. M.G Pap. Carc Lungs Riddled Metas	(1) 10x10x10 1000 Cmm Dec. 1 '14 11x11x10 1210 Cmm (2) ———	(1) 50x50x40 100000 Cmm (2) 12x6x6 432 Cmm	Tumors	Dec. 1 '14 Jan 10 '15	Feb 7 '14 Jan. 10 '15 337 days	(1) 210 in 93 98790 in 40 (2) 432 in - 99222 in 40	(1) 2.25 Reprod 2469.75 Non-Reprod (2) 10.8 2480.55 Non. Reprod
9712	Jan. 5 '15 Apl. 28 '15 113 days	4	18	1	Lf. Ax. M.G to Base Ear Hem. Ad. Carc	10x10x10 1000 Cmm Apr. 10 '15 20x15x12 3600 Cmm.	40x40x35 56000 Cmm	Tumor	Apr 10 - Apr. 28 '15	June 10 14 Apr. 28 '15 322 days	2600 in 95 52400 in 18	27.36 Reprod 2911 11 Non Reprod.
9996	Oct 24 '14 Mch 31 15 158 days	5	24	1	Rt. Sub-Ax. M.G. Tub. Pap Carc Lungs Replaced Metas	8x8x8 512 Cmm Feb 11 '15 10x8x8 640 Cmm	20x15x20 6000 Cmm	Lung Tumors Retained Fetus Gen. Sepsis	Feb. 11 - Mch. 31 '15	July 7 '13 Mch. 31 '15 632 days	128 in 110 5360 in 48	1.16 Reprod 111.66 Non-Reprod

The youngest of these mice was 282 days, the oldest was 1024 days, the average age was 465 days or 1 year, 3 months, 10 days, the majority of the mice being in the height of the reproductive age at the time of the origin of their tumors. Every mouse from the youngest to the oldest shows a tremendous increase of tumor growth after she ceased to be reproductive.

Note the three cases of mammary gland sarcoma which have been introduced here for purposes of comparison.

1. Female 7454, who had at death a spindle-cell sarcoma right to left axilla 42 x 40 x 30 mm. (50,400 cmm.), grew only 3.35 cmm. of tumor daily during her reproductive period of 217 days after the appearance of tumor, while she grew an average of 1946.88 cmm. of tumor daily in the 25 days she was non-reproductive (Chart 6).

2. Female 8889 had at death a tri-nodular tumor 50 x 30 x 30 mm. (45,000 cmm.), this being a spindle-cell sarcoma between two alveolar tubular carcinomas, the whole growth extending from the left to the right axilla and down to the right forefoot. This mouse showed an average daily tumor growth of 56.91 cmm. during her reproductive period of 123 days, while the rate of growth jumped to an average daily rate of 2643.42 cmm. during the fourteen days she lived after she ceased reproducing (Chart 6).

3. Female 10,015 with a right inguinal alveolar sarcoma and a right axillary partly cystic-squamous, partly papillary adenocarcinoma yielded the following data: The sarcoma grew an average of 1.66 cmm. daily during the 120 days she was reproducing, while an average daily growth of 461.53 cmm. succeeded in the 13 days she was non-reproductive. The carcinoma developed entirely during her non-reproductive period at the daily rate of 15.38 cmm. (Chart 7).

The papillary adenocarcinomas showed the same relative rates of growth during the reproductive and the non-reproductive periods. Note female 7536, (chart 6), average daily growth 0.79 cmm. while reproductive, 77.33 cm. while non-reproductive.

The problem of this retardation of tumor growth during the period of active reproduction is a complex one, and it is necessary to eliminate the other obvious factors in such delay.

Let me repeat at this point what I already have published frequently, viz: the mice in this laboratory have behind them many generations of hygiene as perfect as the most rigid care can secure. They come of strains whose members are vigorous, sleek, active, well grown, long-lived, and highly reproductive. The individuals taken for this study are among the most vigorous mice in the laboratory. A glance at the number of litters and the number of young they bore after the appearance of their tumors will attest this vigor to anyone who has ever tried the work of breeding mice with spontaneous tumors. These mice have been kept as free from other diseases as it is possible to keep them. Nevertheless many of the cases are complicated by causes of death other than tumor, and this is obviously one of the factors in the varying amount of tumor grown by different individuals. The relation of other diseases to the amount of tumor growth is a subject much too large for the confines of this paper. It will be treated in detail in a forthcoming communication; but let me at this time point out a few facts in this connection as shown by the individuals listed in these charts.

Female 3621 (chart 5) died of an infection characterized by extensive liver necrosis. She lived 22 days after the birth of her last litter (born dead) and grew during this time only 976 cmm. of tumor, 44.36 cmm. daily average. She was 1 year, 1 month, 12 days old, scarcely past the prime of reproductive life in mice. Compare this amount of tumor growth with that of female 8889 (chart 6) who was just 15 days younger and who died of tumors uncomplicated by any other disease. This latter grew 37,008 cmm. of tumor in the 14 days she lived after her last litter, an average daily growth of 2643.42 cmm.

Female 4554 (chart 5) died May 15, 1913, of general sepsis from infected uterus with an undelivered fetus remaining from her last litter, born dead April 30. She was 282 days (9 months, 12 days) old, in the prime of reproductive life. She grew only 784 cmm. of tumor in the 15 days she lived after her last litter, or an average daily growth of 52.26 cmm.

Compare this amount of tumor growth with that of female 9172 (chart 8), fifty-five days older, where the cause of death was not complicated by any other disease than tumor. This latter female grew 99,222 cmm. in the 40 days she lived after the birth of her last litter, an average daily growth of 2480.55 cmm.

CHART 9

NON-REPRODUCING FEMALES —

AGE IN DAYS	AVERAGE DAILY TUMOR GROWTH CMM	CAUSE OF DEATH
216	2270.74	TUMOR
227	1654.73	TUMOR
253	1626.66	INFLAM. LUNGS
272	1437.89	TERM. INFECT.
299	1439.52	CHRONIC NEPH.
311	1141.42	EDEMA LUNGS
317	1127.48	AMYLOIDOSIS
323	1233.80	TERM. INFECT.
325	1687.77	TUMORS
352	1119.36	TERM. INFECT. PULMON. INFECT
354	1430.	ATROPHIED ORGANS FROM TUMOR PRESSURE
354	937.50	TERM. INFECT.
356	821.78	PULMON. INFECT
359	947.23	TUMOR
361	1601.34	CHRONIC NEPH.
371	602.27	AMYLOIDOSIS
381	654.71	HYPERTROPHIED HEART
390	1214.18	GASTRIC HEM.
392	1125.33	TUMORS
396	977.29	CHRONIC NEPH.
409	632.58	TUMORS
416	730.14	HYPERTROPHIED HEART
428	1115.53	TERM. INFECT.
488	288.78	GASTRIC HEM.
489	779.75	TERM. INFECT
544	595.48	TUMORS
549	141.33	ABSCESS IN LIVER TERM. INFECT.
558	267.46	TUMORS
669	294.16	CHRONIC NEPH.
803	87.29	TERM. INFECT.

Female 9053 (chart 6), only 293 days old, died of tapeworm. She grew only 548 cmm. of tumor in the 6 days she lived after her last litter, an average daily growth rate of 91.33 cmm.; while female 7536 (chart 6), who died of tapeworm and nematodes, grew an average of only 77.33 cmm. of tumor in the 18 days she lived after her last litter. She was 351 days old.

Compare these two with female 15768 (chart 8), 352 days old, who had only a slight chronic nephritis in addition to her tumors. She produced a daily average of 1194.85 cmm. of tumor in the 20 days she lived after her last litter (born dead); compare also with female 15,990 (chart 7) of about the same age, 348 days, who had a brief acute pulmonary infection. She produced an average of 1448.18 cmm. of tumor daily.

In every tumorous mouse that I have handled whose disease was complicated by tapeworm or nematodes or both, tumor growth has been seriously interfered with, as it has been seriously interfered with by any other rapidly destructive type of complicating disease.

Other diseases, then, are one factor modifying the rate of tumor growth.

CHART 10

Tumors Only	
Age in Days	Daily Rate Tumor Growth cmm.
216	2270.74
227	1654.73
325	1687.77
359	947.23
392	1125.33
409	632.58
544	595.48
558	267.46

Age, as is rather generally admitted, is evidently another factor which modifies the rate of tumor growth.

I have drawn up a chart in age sequence, of the thirty females not reproducing after the appearance of their tumors (Chart 9).

The youngest mouse here, 216 days old, showed the largest average rate of tumor growth, viz: 2270.74 cmm. daily, while the oldest mouse, 803 days, produced the smallest daily average of tumor growth, viz: 87.29 cmm. The intermediate mice show a fairly well graduated decrease in average daily tumor growth as the age increases, the relatively few irregularities being explained at least in part by the complicating diseases. For if we take those that died of tumors only, shown in chart 10, the sequence is pretty regular from the youngest, 216 days old and with a daily rate of 2270.74 cmm., to the oldest, 558 days old with a daily rate of 267.46 cmm.

For those dying of a complicating pulmonary infection, the sequence is entirely regular, the youngest, 253 days, growing 1626.66 cmm. daily, while the oldest, 356 days, grew 821.78 cmm. daily.

Of those dying of a terminal infection the sequence again is notably regular from the youngest, 272 days, with an average

CHART 11

Pulmonary Infection	
Age in Days	Daily Rate Tumor Growth
253	1626.66 cmm
311	1141.42
352	1119.36
356	821.78

CHART 12

Terminal Infection	
Age in Days	Daily Rate Tumor Growth
272	1437.89 cmm
323	1233.80
352	1119.36
354	937.50
428	1115.53
489	779.75
549	141.33
803	87.29

growth of 1437.89 cmm. to the oldest, 803 days, with an average rate of 87.29 cmm.

For those dying of chronic nephritis the sequence is fairly regular, and for those dying of gastric hemorrhage, entirely so.

CHART 13

Chronic Nephritis	
Age in Days	Daily Rate Tumor Growth
299	1437.89 cmm
361	1601.84
396	977.29
669	294.16

CHART 14

Gastric Hemorrhage	
Age in Days	Daily Rate Tumor Growth
390	1214.18 cmm
488	288.78

In the non-reproducing females, then, there appear only two complicating factors outside of the general metabolic condition of the mouse and the fact that it is at the height of the reproductive age but is not reproducing, viz: the age of the mouse, and those diseases other than tumor which cause death.

Bearing in mind, then, that both age and a destructive complicating disease modify the results, the tremendous amount of tumor uniformly grown by these non-reproducing mice (of reproductive age) stands out with extreme clearness. The normal

course of spontaneous tumors in mice of reproductive age which are not being bred, is very rapid. The shortest duration of tumor growth in these mice was 19 days; the longest was 54

CHART 15

REPRODUCING FEMALES

Age in Days.	Average Daily Tumor Growth while reproductive cmm	Average Daily Tumor Growth while non-reproductive cmm	Cause of Death
282	1.86	52.26	Infected Uterus (Dead Fetus) Gen. Sepsis
293	.32	91.33	Tapeworm Chr. Neph.
322	27.36	291.11	Tumor
333	4.97	332.50	Chr. Neph.
337	2.25	2480.55	Tumor
337	2.94	1102.19	Leukemia.
341	6.11	759.74	Tumor
348	15.79	1488.18	Pul. Infect with Hem.
351	.79	77.33	Nematodes Tapeworm
352	9.57	1194.85	Chr. Neph
387	6.77	442.76	Hydrothorax Pul. Infect
388	1.66	476.91	Liver Abscess Amyloidosis.
392	56.91	2643.42	Tumor
407	.19	44.36	Liver Necrosis
425	2.95	127.80	Term. Infect.
437	37.65		Hyp. Heart Hydrothorax
443	2.28	513.12	Tumor
459	3.35	1946.88	Tumor
485	2.80	170.87	Hem. Ovary Lung Tumor
488	1.12	1040.64	Tumor
509	3.41	201.22	Chr. Neph.
510	2.52	131.36	Hyp. Heart Lung Tumors
569	1.02	299.44	Intest. Infect.
576	2.21	351.42	Acute Neph.
580	3.87	1178.62	Tumor
632	1.16	111.66	Retained Fetus Gen. Sepsis
655	1.92	215.80	Chr. Neph
661	.0	52.30	Hem. Ovary
740	.77	8.25	Hyp. Heart Hydrothorax
1024	2.82	143.43	Chr Neph

days; the average was 35 days, or 1 month, 5 days. These tumors grow to a great size, frequently being as large as the body of the mouse itself. In over 70 per cent of the cases multiple tumors arose, there being but 8 cases with one tumor only; 8 cases with two tumors; 9 cases with three tumors; 4

cases with four tumors; and one case with six tumors. 13 cases, or nearly one-half, showed pulmonary metastases.

In the age chart of the reproducing females the influence of age upon the amount of tumor growth is much less apparent (Chart 15). Even when charted in age periods of 100 days, the influence of age upon tumor growth is much less evident in the reproducing females than in the non-reproducing (Chart 16).

This is in part explained by the complicating causes of death, such as tapeworm and nematodes in the digestive tract, general sepsis from an infected uterus with dead retained fetus, etc. For if we take the females dying of carcinoma only, eliminating

CHART 16

AVERAGE DAILY GROWTH CMM.

	Non-Reprod.	Reproductive	
Age Periods —		Non-Reprod. Period	Reprod. Period
200-300 Days	1685.70	71.79	1.09
300-400 Days	1108.13	1053.59	12.28
400-500 Days	709.35	640.11	7.19
500-600 Days	334.75	435.21	2.60
600-700 Days	294.16	126.58	.69
700-800 Days		8.25	.77
800-900 Days	87.29		
1000 Days		143.43	2.82

CHART 17

REPRODUCING FEMALES. DYING OF CARCINOMA ONLY

Age in Days	Tumor Growth while reproducing	Tumor Growth while not reproducing
322	27.36 cmm	2911.11 cmm
337	2.25	2480.55
341	6.11	759.74
443	2.28	513.12
488	1.12	1040.65
580	3.87	1182.00

those that died of more rapidly growing sarcoma, or of complicating destructive diseases like tapeworm and general sepsis, we get a better sequence (Chart 17).

Even here, however, there is evidence of *some other factor affecting the amount of tumor grown by reproducing females.*

If we chart these same carcinomatous females in sequence of the number of young borne while they were tumorous, we get almost a perfect sequence, the one exception apparently being accounted for by age difference. In these reproducing females, then, *the number of young borne after they are tumorous, seems also to be a factor in determining the amount and rate of tumor growth.*

Bearing in mind these three factors, age, other complicating diseases, and the number of young borne, note the tremendous increase of tumor growth in these mice after they cease reproducing.

The normal course of these spontaneous mammary gland tumors in mice which are constantly reproducing, is very slow. In many cases the tumor scarcely grows at all during this period, one tumor showed no growth whatever during 45 days of reproduction. The lowest daily rate of tumor growth was 0.19 cmm.; the highest daily rate was 56.91 cmm.; the average rate was 7.75 cmm. The duration of the tumor is greatly prolonged by reproduction, the mouse sometimes living nearly a year after the appearance of her tumor while she bears many litters of young. The shortest duration of tumors in these mice was 58 days, the longest 347. The average duration was 177.87 days or 5 months, 27.9 days as compared with 1 month, 6.4 days in the non-reproducing females of about the same age.

CHART 18

Reproducing Females Dying of Carcinoma Only

No. of Litters	No. of Young	Rate of Tumor Growth while not reproducing	Age in Days
4	15	2460.55 cmm	337
4	18	2911.11	322
7	20	1182.00	580
7	23	1040.65	488
4	25	759.74	341
8	31	513.12	443

The smallest number of young borne by these mice after their tumor appeared was 7, the largest number was 32; the average was 20.

When these females cease reproducing the tumors grow with great rapidity, the mouse frequently living only a few days after the birth of her last litter. The shortest period after the last litter was less than 1 day, the longest was 128 days (in a mouse 2 years, 9 months, 24 days old). The average duration of the tumor in the non-reproductive period was 37 days.

This average duration period is almost exactly the period of duration of the tumor (35 days) in the thirty non-reproductive females of almost the exact age, which therefore make a valuable control in this study of the reproducing females. During this brief period, often only 8 or 10 days, the tumor grows to many

times the size attained during the entire period of reproduction, averaging nearly six months.

The lowest daily rate of tumor growth during the non-reproductive period was 8.25 cmm. (in a mouse nearly 2 years old). The highest daily rate was 2911.11 cmm. (in a very young mouse about 10 months old); the average rate was 686.34 cmm., considerably less than the average daily rate of the non-reproducing females of nearly the exact average age (999.45 cmm.) whose tumors were of nearly the exact average duration (35 days).

This emphasizes again the probability that the number of young borne after the appearance of the tumor is a factor in the amount of tumor grown after the reproductive period is past,

CHART 19

Averages		
	Non-Reproductive ♀	Reproductive
Age	415 Days (1 yr. 1 mo. 20 Days)	465 Days (1 yr. 3 mo. 10 Days)
Duration of Tumor	1 mo. 6.4 Days	5 mo. 27.9 Days
Daily Rate of Tumor Growth cmm	999.42	686.34 Non-Reprod Period 7.75 Reproductive Period.

and links these two modes of growth, the growth of embryos and the growth of tumor in a very close relationship. Multiple tumors appeared much less frequently in these reproducing females than in the non-reproducing. There were 15 cases of single tumor growth, as compared with 8 cases in the non-reproductive females. There were 9 cases with two tumors, as compared with 8 in the non-reproducing females, 5 cases of 3 tumors as compared with 4 in the non-reproducing, while 4 was the highest number of tumors occurring in any one of these reproducing mice, there being but one such case. 50 per cent of these cases showed multiple tumors as compared with over 70 per cent of multiple tumors in the non-reproducing females. Ten cases, or one third, showed lung metastases.

Chart 19 shows the average age, duration of tumor growth, and rate of tumor growth of the 2 sets of mice, the non-reproducing and the reproducing.

SUMMARY

In handling large numbers of mice with spontaneous tumors there is forced upon the observer from the very first the great difference in the rate of tumor growth in the non-reproducing and in the reproducing females.

The same difference is noted in the rate of tumor growth in the non-reproductive and in the reproductive periods of the same female.

For this study thirty each of non-reproducing and of reproducing females with spontaneous tumors were selected. The tumors were all of the same type and of the same organ (with a few exceptions for purposes of comparison), viz: alveolar tubular carcinoma of the mammary gland, of which a daily observation is easily made.

Without exception, the amount of tumor grown by a female while reproductive was strikingly less than during her non-reproductive period.

Again, the amount of tumor grown by reproducing females was strikingly less than that grown by non-reproducing females.

The normal course of these spontaneous tumors in mice that are not bred is very rapid, the mouse rarely living over six weeks and often less than a month after the appearance of her tumor. The tumors grow to a great size, frequently being as large as the body of the mouse.

When, however, these tumorous mice are bred, the tumor scarcely grows at all during the reproductive period. The duration of the tumor is greatly prolonged, the mice frequently living nearly a year after the appearance of their tumors, during which time many bear from six to eight litters aggregating from twenty to thirty-two young. When the mouse ceases reproducing, the tumors grow with tremendous rapidity and to great size, the female frequently surviving only six or eight days after the birth of the last litter. During this brief period the tumor grows to many times its size at the date of the last litter.

In brief, during the six or eight days a mouse is non-reproductive, she grows enormously more tumor than during the eight months or a year while she is reproductive, the daily rate of

tumor growth being far in excess of the daily rate during the reproductive period.

Two other factors must be taken into consideration, viz: the age of the mouse, and other complicating causes of death. Generally speaking, the younger mice show a higher daily rate of tumor growth than do the older mice.

Again, complicating diseases such as tapeworm and nematodes in the digestive tract, or any other highly destructive disease, greatly retard tumor growth; and *the number of young borne after the appearance of tumor is also a factor in reproducing females.* But when these factors have been eliminated two facts stand out with startling clarity and cannot be gainsaid, viz:

1. Reproducing females grow much less tumor than do non-reproducing females of the same approximate age and general metabolic condition.

2. Reproducing females grow much less tumor while they are reproductive than they do while they are non-reproductive; in other words, *when a mouse is producing embryos, she is not producing tumor in anything like the amount which she grows while non-reproductive.* Multiple tumors are more common in the non-reproducing than in the reproducing females, the figures being over 70 per cent compared with 50 per cent.

In striking contrast to these results is the relation between pregnancy and the infections common to mice. If an infected mouse is bred, instead of having the infection held off for a year or more while she bears young, she is unable to produce any young at all and speedily dies of her infection. Or if a pregnant mouse contracts an infection, she rarely brings her young to birth.

The results of this study bring out with striking force the close relation between tumor production and the production of young, showing them to be two closely related modes of growth.

CONCLUSIONS

1. Cancer and reproduction, both being growth processes, draw upon the same energy residuum and are made possible by the same food. Hence the food and energy used by one are withheld from the other.

2. Therefore (a) if the female is *constantly pregnant*, energy and food are withheld from the tumor and it grows with extreme slowness. (b) If there is a hiatus between pregnancies, or a termination of pregnancy, the energy which was running into reproduction is released and diverted into tumor which grows very rapidly. (c) If tumor growth considerably antedates impregnation, the currents of energy are already being used for tumor growth and are with difficulty diverted for pregnancy, probably never wholly so.

3. Hence, when a female is well advanced in tumor growth before impregnation there are rarely any offspring brought to birth. When offspring are delivered they are few, small, undernourished, and rarely suckled (which in mice means there is no lactation).

4. When tumor growth is not interfered with by pregnancy, it is (a) extremely rapid in mice which are young, well nourished, and vigorous; (b) less rapid in mice older or less vigorous, or less nourished; (c) very slow in mice which are old, feeble, under nourished, or afflicted with a destructive complicating disease.

5. Another point which shows the close relation between the growth of embryos and the growth of tumor is the great frequency with which breast tumors are nearly synchronous with delivery. Hyperstimulation of any tissue seems to originate cancer of those tissues in individuals of cancer tendency; hence the intense stimulation incident upon lactation tends to originate cancer of the breast in individuals of breast cancer tendency.[3]

6. The prolonged hiatus between pregnancies greatly complicates the study of the relation between pregnancy and tumor growth in the human species. During this prolonged hiatus the tumor may draw off the energy which would have continued to be used in reproduction if the pregnancies were not widely separated, just as is the case in mice kept constantly impregnated. This would account for any apparently conflicting testimony in human experience as compared with these studies.

[3] This subject will be more fully treated in a forthcoming paper.

The factors are not subject to control in the attempt to study the relation between reproduction and tumor growth in the human species, and the conclusions have to be drawn without knowledge of complicating factors. The real relation between these two can be disclosed only in the experimental laboratory, where the factors are all known and are under control.

The experimental evidence shows a very striking relation between these two modes of growth, the production of young and the production of tumor; moreover, it shows the same relation between the production of young, and the growth of *all types* of mammary gland tumors.

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THE RELATION OF INBREEDING TO TUMOR PRODUCTION: STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

XIII. PROBLEMS IN THE BEHAVIOR OF TUMORS

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Throughout the range of cancer research, there seems to be no other point so frequently and so completely misunderstood as the subject of inbreeding in its relation to the incidence of tumor. Since the beginning of this series of publications, these researches into the problem of the inheritability of cancer have been met by the statement that "inbreeding increases the number of tumors in a strain," or that "inbreeding is responsible for the high incidence of tumors in a strain of mice, and consequently the demonstration of the inheritability of cancer for mice has no bearing upon the human species, since the latter is not characterized by inbreeding."

The latest and most conspicuous example of this misapprehension of biological procedure and fact appears in Ewing's recent compendium "Neoplastic Diseases," (1) in which he states that "Bashford *attempted* by inbreeding to *intensify* the hereditary influence," and that "Slye *has proven* that *inbreeding* of *tumor-bearing animals greatly increases* the *incidence* of tumors." With this misinterpretation, he dismisses all the exact indisputable experimental evidence for the inheritability of tumors in general and of all types, including cancer, in particular.

It is precisely because inbreeding does not characterize the human species that it is impossible to make any even reasonably complete or accurate study of the inheritability of cancer in

that species, and hence that experimental evidence becomes absolutely necessary as it is impossible to *prove* the inheritability of any character without inbreeding. Mendel in his work with peas was not trying to increase roundness or ovalness, yellowness or greeness, tallness or shortness, or any other quality of peas. He was trying to find out whether these characters were hereditary, and in order to find out whether they were hereditary, he *had to inbreed his peas.*

Cuenot (2) when he crossed the albino mouse with the house mouse, was not trying to increase albinism or greyness or any other quality of mice, he was trying to find out whether pigmentation and lack of pigmentation are hereditary; and in order to do this he *had to inbreed his mice.* He did not thereby *increase* albinism, or *increase* the agouti coat, or *increase* any other character. He demonstrated that pigmentation and albinism are inheritable and in demonstrating that pigmentation and lack of pigmentation are inheritable in mice (whose pigment is melanin) he demonstrated that these characters are inheritable also in guinea pigs, or in rabbits, or in man (in all of which species the pigment is melanin).

Neither Bashford nor Slye has "*attempted to prove*," or "proved that inbreeding increases the incidence of tumors." Neither have Lathrop and Loeb or Little and Tyzzer attempted to demonstrate any such absurdity. All of these experimental workers in cancer have attempted to prove or disprove the inheritability of cancer and they have *all inbred their animals,* because only by inbreeding can you analyze a strain. By inbreeding one discovers what is in a strain, *he does not "increase" or "intensify" anything.*

For the last ten years there have appeared from this laboratory many charts showing the exact genealogical data of strains of mice (families) in which cancer has been transmitted through generation after generation without a break and *in strikingly perfect Mendelian ratios.* At the same time other charts have been published showing the exact genealogical data of families of mice in which there has never been any tumor of any sort, through generation after generation, where the mice have been handled with identical technique *including constant inbreeding.*

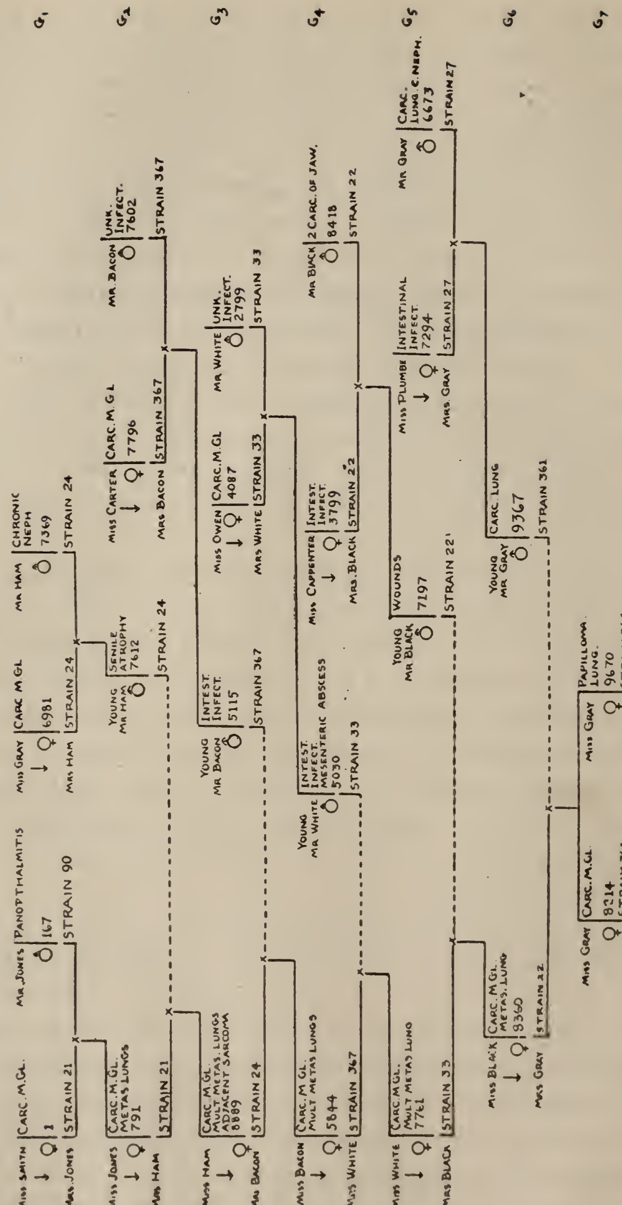
The genealogical charts which have appeared in previous reports from this laboratory have all involved the inbreeding of filial generations of offspring from hybrid crosses, because only so is it possible to find out accurately *just what characters are transmitted in a hybrid cross* and *in just what ratio they are transmitted.* This is the method of all biological procedure in the study of heredity. There is no other method. Just as the analytical chemist does not analyze his unknown by throwing in more unknowns, so the analytical biologist cannot analyze his strain by throwing in more unknowns (by hybridizing) but must inbreed. For the benefit, however, of those who do not comprehend or accept this method, I have drawn up a chart of matings made in this laboratory, which is the duplicate of human mating, and in order that no point in it may escape observation I have decorated it with human nomenclature (Chart 1).

This chart is the exact duplicate of human genealogical charts, in that it contains no inbreeding and not even any consanguineous matings. Miss Smith (female 1) is mated with Mr. Jones (male 167); the offspring of this mating, viz., Miss Jones (female 791) is mated with young Mr. Ham (male 7612) whose medical genealogy is indicated in the chart; etc. Through seven generations one daughter of each pair, is in turn mated with a wholly unrelated male, whose medical genealogy is shown. Every mating in the chart *is a hybrid cross*, just as is the case in all human matings.

Note (1) that carcinoma of the mammary gland entered in the parent female who began this family, viz. Miss Smith (female 1); (2) that the male parent in each generation was the immediate offspring of a cancerous progenitor; (3) that the daughter in every generation exhibited carcinoma of the mammary gland; (4) that lung tumors entered on the male side in the fifth generation; (5) that these lung tumors were exhibited by each succeeding generation (generations 6 and 7).

It must not be inferred from this chart that mammary gland carcinoma can be transmitted only by the female, or lung tumor only by the male. They happened to be so transmitted in this family. But there are many other families in which mammary

CHART I


G1
G2
G3
G4
G5
G6
G7
Miss Smith
Mrs. Jones
Carc. M.Gl.
1
Strain 21
Mr. Jones
Panopthalmitis
167
Strain 90
Miss Gray
Mrs Ham
Carc. M Gl
6981
Strain 24
Mr Ham
Chronic Neph
7369
Strain 24
Miss Jones
Mrs Ham
Carc. M.Gl.
Metas Lungs
791
Strain 21
Young Mr Ham
Senile Atrophy
7612
Strain 24
Miss Carter
Mrs Bacon
Carc. M.Gl
7796
Strain 367
Mr. Bacon
Unk. Infect.
7602
Strain 367
Miss Ham
Mrs Bacon
Carc. M.Gl.
Mult. Metas. Lungs
Adjacent Sarcoma
8889
Strain 24
Young Mr Bacon
Intest. Infect.
5115
Strain 367
Miss Owen
Mrs White
Carc. M.Gl
4087
Strain 33
Mr White
Unk. Infect.
2799
Strain 33
Miss Bacon
Mrs White
Carc. M.Gl.
Mult Metas Lungs
5844
Strain 367
Young Mr White
Intest. Infect.
Mesenteric Abscess
5030
Strain 33
Miss Carpenter
Mrs. Black
Intest. Infect.
3799
Strain 22
Mr Black
2 Carc. of Jaw.
8418
Strain 22
Miss White
Mrs Black
Carc. M.Gl.
Mult Metas Lung
7761
Strain 33
Young Mr Black
Wounds
7197
Strain 22
Miss Plumb
Mrs. Gray
Intestinal Infect.
7294
Strain 27
Mr Gray
Carc.
Lung. C. Neph.
6673
Strain 27
Miss Black
Mrs Gray
Carc. M.Gl.
Metas. Lung
8360
Strain 22
Young Mr Gray
Carc. Lung
9367
Strain 361
Miss Gray
Carc. M.Gl.
8214
Strain 361
Miss Gray
Papilloma Lung.
9670
Strain 361

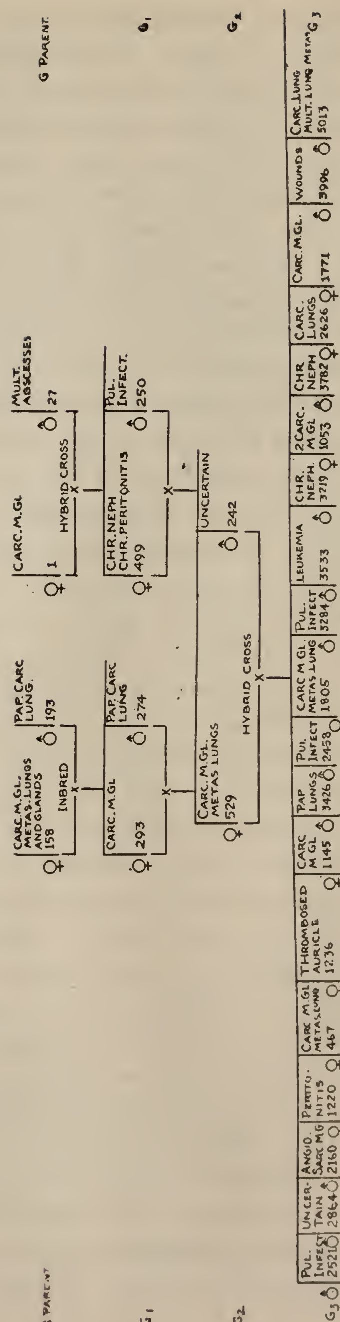
gland carcinoma was transmitted by the male (note chart 2, strain 146, branch II) and lung carcinoma by the female. Throughout the work of this laboratory no allelomorphism has been demonstrated between sex and type or location of tumor, except, of course, in the sex organ tumors. There is not apparently any allelomorphism even between sex and mammary gland tumors, since this laboratory has yielded a considerable number of males with mammary gland tumors and males who have transmitted mammary gland tumors. The great preponderance of this location of tumor in the female is presumably due to the greater frequency of hyperstimulation of these tissues in the female. Loeb (3) suggests the possibility that the female may be more potent than the male in the transmission of mammary gland cancer. This suggestion is not borne out by the results in this laboratory, wherein the female is no more prepotent in the transmission of mammary gland carcinoma than she is in the transmission of any other character; and the male is just as potent to transmit mammary gland cancer as he is to transmit a grey coat-color. His female offspring demonstrate the inheritance of mammary gland cancer more frequently than his male offspring for the reason stated above, viz., the more frequent chronic irritation of mammary gland tissues in the female. This subject will be discussed more fully in a forthcoming paper.

Innumerable matings of this sort (i.e. like human matings) can be made and their charts published, but it is useless to waste many cancerous mice in such crosses, because they are impossible of accurate analysis. It is best to follow the exact method whereby through analysis one can become acquainted with the intrinsic characters of individual mice and of strains of mice. It is then possible to manipulate the characters of the individual mice or of the strains of mice with a certainty of outcome; entirely eliminating the uncertainty attendant upon the so-called "statistical method," which in reality is not a method at all.

Let us analyze, therefore, some of the charts which have been misunderstood to demonstrate that "inbreeding increases tumor." These charts have been published in reports from this laboratory to which Ewing refers (4), (5), (6). Chart 2 shows the first filial

CHART 2

STRAIN 146 WITH ANCESTRY.



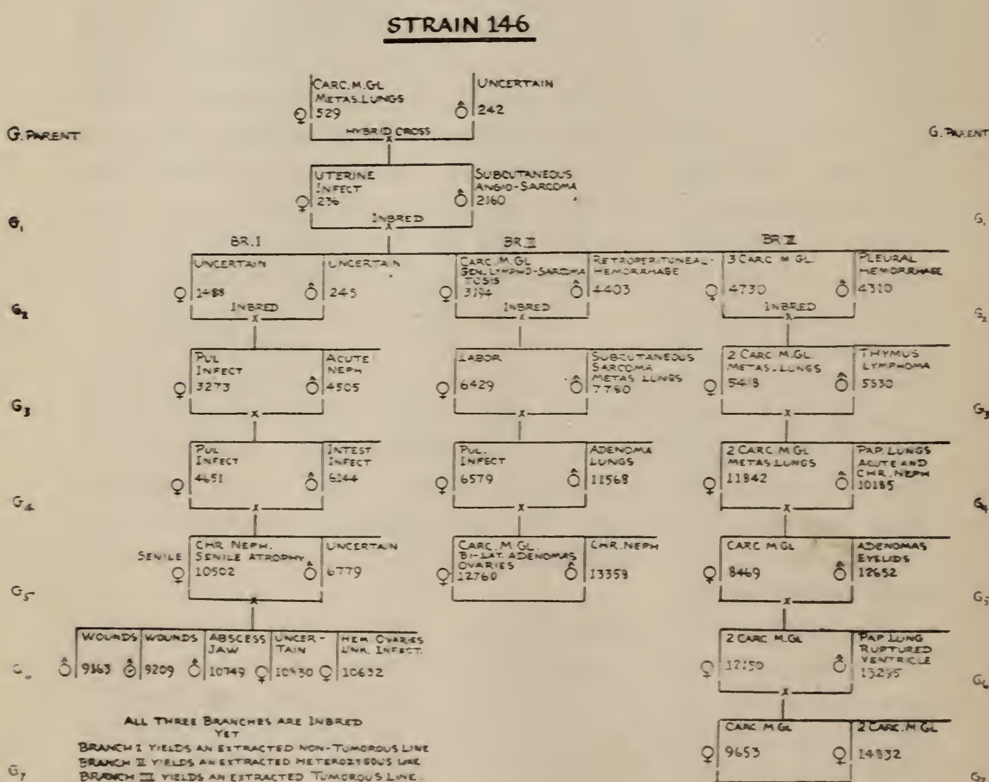
generation of strain 146, with its ancestry for three generations. This strain is the product of a *hybrid cross*, female 529 being in no way related to her mate, male 242. Female 529 died of carcinoma of the mammary gland with metastases in the lungs. Her mother and her grandmother, females 293 and 158, both died of carcinoma of the mammary gland. Her father and her grandfather, males 274 and 193, both died of carcinoma of the lung. She came of a family which carried in all its branches 100 per cent of carcinoma. Her ancestry for two generations was inbred, and we therefore know her to be an extracted cancer-bearing individual, capable of carrying cancer into any strain into which she is mated, with the same certainty with which she will carry albinism. We have then in her, by this method of inbreeding, not an unknown factor, but one whose influence in any compound of which she forms a part can be predicted both in the matter of color and of disease; and whose influence has actually been demonstrated to be just as predicted, both in regard to albinism and in regard to cancer. Her mate, male 242, died of an undetermined disease and had no tumor. His father and his mother, male 250 and female 499, did not exhibit tumor of any sort; his grandmother, female 1, however, died of carcinoma of the mammary gland; his grandfather, male 27, of multiple abscesses without tumor. He comes, then, of a *hybrid*, not an inbred line, with carcinoma of the mammary gland two generations back.

His mating with female 529 is a *hybrid cross* (*not inbred*). Note the number of tumorous individuals in the first *hybrid* generation, viz., nine out of nineteen (of the young who lived to cancer age), the almost exact Mendelian expectation from the mating of an extracted cancerous female with a male heterozygous to cancer (that is carrying it potentially, but not himself developing the disease). A hybrid strain then, which carries 47 per cent of tumor in its first generation (*without inbreeding*) is the product of this cross.

Chart 3 carries on three branches of this hybrid strain 146, and shows what results followed *inbreeding* in each of the above branches.

We have here this same female 529, an extracted cancer-bearing female, mated with male 242 (a heterozygote). The two first-generation hybrids mated in this chart are female 236, a non-cancerous female who died of uterine infection, and male 2160 who died of a subcutaneous angiosarcoma, probably of the

CHART 3



mammary gland. Branch I, *although closely inbred* (brother and sister from the same litter in each generation) never yielded a case of any kind of tumor whatever, *i.e.*, it is an extracted non-tumorous line. Branch II, also closely *inbred in exactly the same way* as branch I, yielded a heterozygous line, the tumors being transmitted sometimes through the male and

sometimes through the female. (Note the mammary gland tumor transmitted through the male in branch II.) While branch III, also *inbred in exactly the same way* as branches I and II, yielded an extracted tumorous line, nearly 60 per cent of these tumors being carcinoma of the mammary gland.

Here are three branches then of the same hybrid family from a cancerous mother, all *inbred in exactly the same way*, yielding three totally different results, *thereby completely eliminating inbreeding as a determining influence* in the incidence or the ratio of tumor production. Moreover, these results are exactly in accordance with Mendelian law.

Ewing (7) states that "the Mendelian characters noted in the heredity of some pathological conditions have not been traced with tumor." All the charts in this paper, and all other charts put out from this laboratory clearly show the "Mendelian character of tumor inheritance." He states further: "the predisposition might be congenital without being hereditary." It is an axiom in genetics that *characters which segregate out*, as do cancer and non-cancer, are hereditary and not congenital. If we do not admit this, no character has ever been proved to be hereditary.

Chart 4 shows strain 164, branch IV, carried out in two families. This is a *hybrid* strain whose progenitors were in no way related. The parent male was a common house-mouse of a strain in my hands many years with no trace of tumor of any sort, although it was *consistently inbred*.

The parent female was a first generation heterozygote of strain 146 (shown in chart 2) who carried cancer into every strain of which she was progenitor, although she herself did not exhibit cancer.

The strain is here shown in two branches *both rigidly inbred*. Branch I is an extracted non-cancerous line, whose members, neither in inbreeding nor in hybridization, ever produced a cancerous offspring. That is, they are proved non-cancerous individuals, whose influence can be predicted in every family into which they are introduced.

CHART 4

STRAIN 164 BRANCH IV

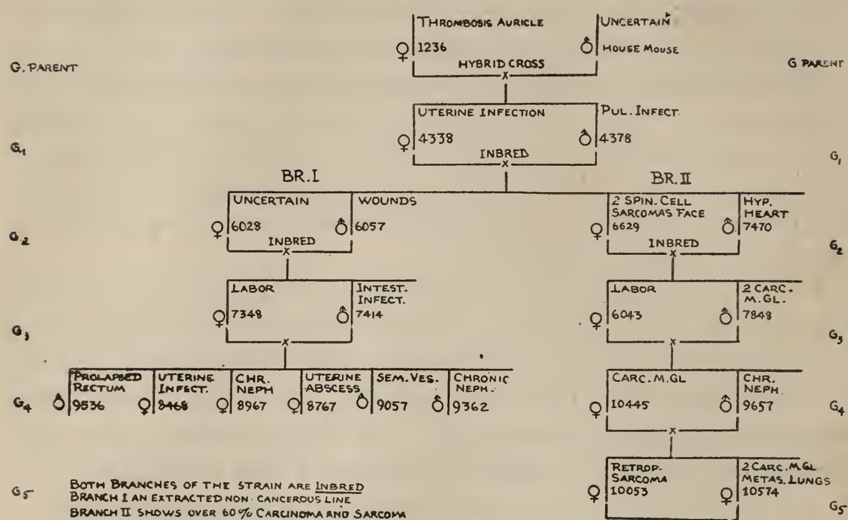
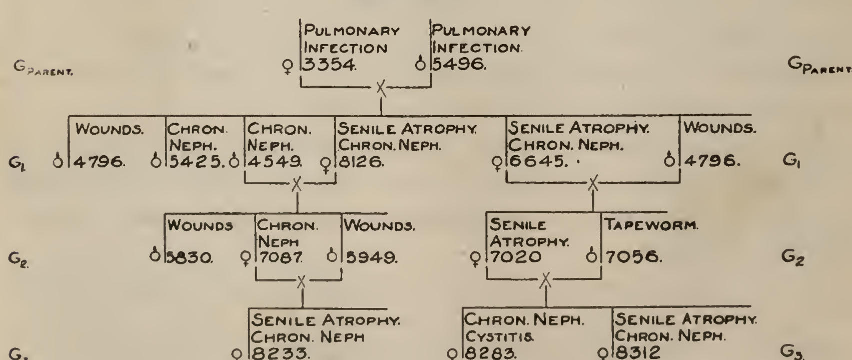


CHART 5

STRAIN 20.



A NON-CANCEROUS STRAIN OF JAPANESE WALTZING MICE
BOTH PARENTS DIED OF PULMONARY INFECTION
THE RESULTING STRAIN SHOWS 0% PULMONARY INFECTION.

Branch II *inbred in exactly the same way* yields over 60 per cent of carcinoma and sarcoma and carries both of these types of neoplasms into every strain into which they are introduced. *Analyzed individuals, then, whose influence can be predicted, are the outcome of the inbreeding method.*

Chart 5 shows strain 20 which has been *closely inbred*, both in the original strain and in its hybrid derivatives, for ten years in my hands, without displaying a tumor of any kind. It is shown in this chart through four generations only; but they are typical of the entire strain and its derivatives, in none of which has cancer or any other tumor ever appeared although the strain has been rigidly inbred.

The foregoing charts are typical. They show conclusively that *inbreeding is not a factor in the increase of tumor*, or in the determination of its incidence; it is merely a method of analyzing a strain in order to determine whether that strain carries cancer or whatever character may be under study.

The real effect of inbreeding upon tumor production seems wholly to have escaped those critics like Ewing who attribute to it any increase in tumor production, although this point was discussed at some length in the Third Report from this laboratory published in March, 1915. It seems advisable, therefore, at this time to demonstrate again as clearly as possible just what is this influence of inbreeding. (Note chart 6, strain 139.)

The parents, female 158 and male 193 (both with carcinoma), were the product of many generations of close inbreeding. They produced only five offspring (compare this with the output of any hybrid cross). These five offspring coming of double cancerous parentage were all cancerous. Four were males and only one a female.

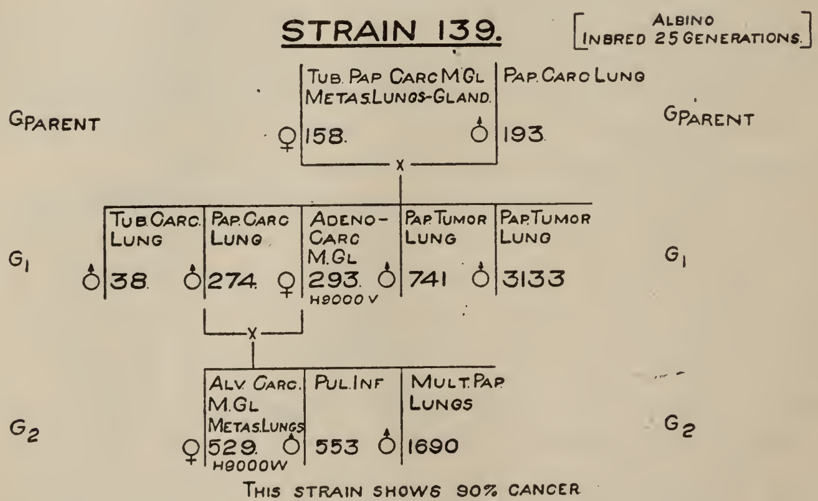
The mating of this one female, number 293, with her brother male 274 (both with carcinoma) produced only three young, two males and one female, all tumorous except the one who died under cancer age, viz., male 553.

The mating of female 529 with her brother male 1690, produced no offspring whatever, and the strain therefore *through close inbreeding was completely eliminated.* Elimination, then, and not increase in tumor, was the result of this inbreeding.

When, however, female 529 who had no young by her brother, was *hybridized* with male 242 (totally unrelated) she became the progenitor of an extremely prolific strain, into which she carried both cancer and albinism. This strain persists in the laboratory to this date, and has in turn furnished the progenitors of many prolific hybrid cancerous strains. This strain, 146, is the one some of whose branches are shown in charts 2 and 3 in this paper.

Again, female 293, first filial generation in strain 139 (chart 6) mated with her brother, male 274 (both cancerous) produced

CHART 6



only three young; mated in turn with her other brothers, male 38 and male 741 (both cancerous) she produced no young. When, however, she was hybridized with male 25 from a totally unrelated strain, she started up a strong prolific line, strain 65, carrying into it both cancer and albinism (shown in two branches in chart 7). Here, again, it was *hybridization which increased the production of tumor; inbreeding which eliminated tumor.*

Instances of this sort could be multiplied in any number desired, but the cases shown in the foregoing charts are entirely typical. *Racially therefore, inbreeding eliminates tumor.*

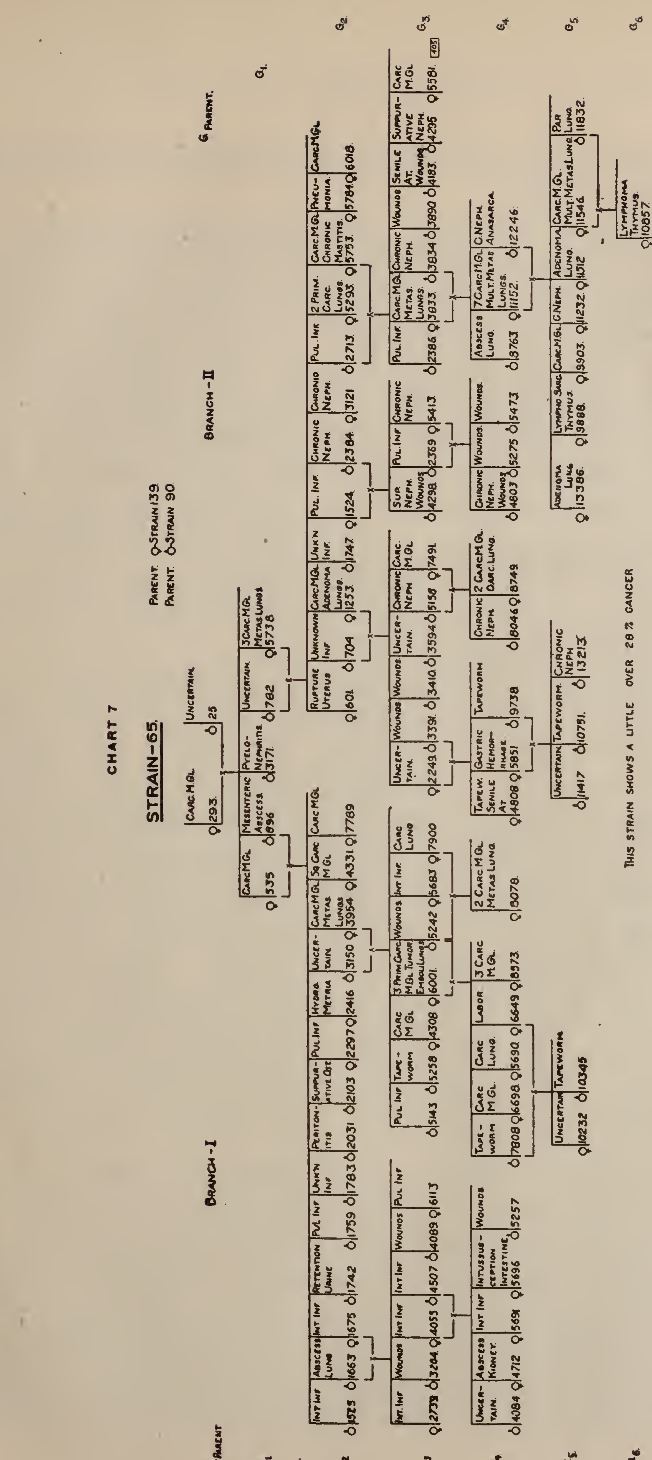


CHART 7
STRAIN-65.
PARENT. ♀ STRAIN 139
PARENT. ♂ STRAIN 90
BRANCH - I
BRANCH - II
THIS STRAIN SHOWS A LITTLE OVER 28% CANCER

The literature yields very little on the subject of inbreeding which is of interest here.

Lathrop and Loeb (9) state: "Continued study of strains of mice in which we have established the tumor rate for earlier generations shows that in the majority of cases the rate remains the same throughout later generations. In most of these strains the constancy in the tumor rate is striking. In a few exceptional cases the rate has increased, but in a considerable number of strains there is a distinct fall." This statement, as well as the explanations offered, indicates that the method was, in the main, mass breeding within the stock and not the controlled selective breeding of individuals. The fall or rise of the tumor rate or its static condition within a strain, would thus be attributable to the reasons assigned by the authors or to any one or combination of many other reasons.

Only by the controlled inbreeding of definite individuals, is it possible to eliminate all other influences determining the rise or fall of the tumor rate. Throughout the work of this laboratory the word *strain* has been used to signify a family arising from a mating between two definite known individuals of known ancestry and known cause of death, the subsequent matings within the strain also being between definite, known individuals of known cause of death. Lathrop and Loeb use the word *strain* as this laboratory uses the word *stock*. These authors say further, "As the result of long continued inbreeding, certain characteristics of a strain change. The strain becomes less prolific and less vigorous, and hand in hand with this change goes a lowering of the tumor rate. This occurred in strain 8 and possibly in other strains."

What these authors find to be true of only one strain, I find to be the law in all strains derived as explained above from a single pair, with all later generations derived from the offspring of this one pair, without the introduction anywhere in any generation of any other member even of the same original stock. This method of mating is the only real inbreeding and the only accurate method of testing the effect of inbreeding. Where this method is followed the most complete and exact analysis

possible is made of the characters transmitted by the original pair.

In the Third Report of this series, published in March, 1915, I stated, "Inbreeding if persisted in eventually wipes out any strain which I have handled" (8). This statement remains unqualified to the present date, there never having been an exception to the rule.

The instances cited in this paper are perfectly typical of all of the hundreds of strains handled in this laboratory. This test has been made consistently for twelve years, and from the facts we are justified in drawing this conclusion: *Consistent inbreeding eliminates any strain.*

It has been the pet argument of workers who maintained that inbreeding had no deleterious effect on a strain, that if perfect individuals were selected for the matings no weakness would be transmitted. A pretty theory but wholly opposed to the facts; for if there were these "perfect individuals" some matings of them would have occurred, and in such strains we should have achieved immortality.

The length of time it takes to eliminate a strain by close inbreeding will depend (1) upon whether or not the stocks crossed produce a fertile hybrid stock. For example, the fancy stocks derived in this laboratory from Mus musculus do not produce a prolific hybrid stock when mated with the Japanese Waltzing mouse. Neither does Mus musculus itself produce a prolific stock when mated with the Japanese Waltzer. Many such strains have been produced in this laboratory, no one of which has ever matched in fertility or vigor of progeny the hybrid strains produced by crossing different stocks where both were Mus musculus derivatives. It will depend, also, (2) upon the vigor of the original pair and whether or not they carry any of the same weaknesses or defects; and (3) upon the vigor of each succeeding pair and what same weaknesses and defects they carry, since they are certain to carry some of the same defects.

If either the original pair or any succeeding pair both carry general defects or weaknesses of the respiratory tract, or of the digestive tract or any organ of this tract, or of the circulatory

tract, etc., the strain will quickly run out, since the offspring receive a double dose of this weakness and inevitably transmit it to all offspring. If both original parents or both parents in any succeeding generation carry cancer or any other type of tumor, the strain will be eliminated relatively quickly, since cancer or any other type of tumor interferes with prolific reproduction (10) and since the offspring receive a double dose of cancer and inevitably transmit it.

Inbreeding then, within a cancer strain, speedily eliminates the strain and instead of increasing cancer as some have inferred, eliminates cancer.

This infertility and general inferiority of the strains derived from crossing the Japanese Waltzer with Mus musculus and its derivatives undoubtedly explains in considerable part if not wholly the results of Little and Tyzzer (11) in their studies in the inheritability of tumor "takes" of their implanted carcinoma, J. w. A., in hybrids of Japanese Waltzers and Mus musculus and its derivatives.

The very fact that J. w. A. does not "take" in Mus musculus or its derivatives, indicates a marked difference in these two races. This same marked difference militates also against a vigorous and prolific strain from the hybridization of these two races.

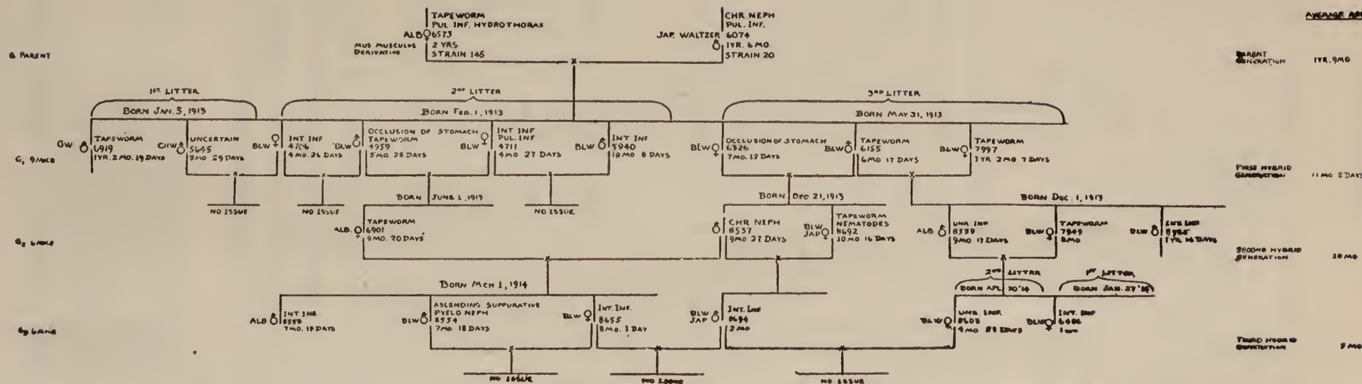
In this laboratory many such crosses have been made. In every case the first generation hybrids have been apparently vigorous mice, inheriting their size from Mus musculus derivatives. But when inbred, they have yielded a very meager output of second generation hybrids, and in no case has it been possible to carry these strains beyond the third hybrid generation, rarely beyond the second. The mice of hybrid generations later than the second are small and apparently entirely infertile when inbred; they are also short lived and relatively inactive.

Chart 8 shows one of these families, strain 479, which is perfectly typical of all the rest.

Strain 479 was produced by mating a Mus musculus derivative, albino female 6573 of strain 145, with Japanese Waltzer male 6074 of strain 20. Female 6573 was a vigorous prolific

CHART 8

STRAIN 479



mouse. Mated within her own strain or hybridized with other Mus musculus derivatives, she yielded prolific sturdy strains.

The parent male 6074, a Japanese Waltzer, mated within his own strain or hybridized with any other Japanese Waltzer strain, produced strains sturdy and prolific as any Japanese Waltzer stock. But these two when cross bred, viz., female 6573 and male 6074, produced a meager strain which died out in the third hybrid generation in spite of every effort to maintain it.

This pair had only 9 young, born in 3 litters; 2 in the first, 4 in the second, 3 in the third. Every possible mating was made of these 9 young, but only 6 second-generation mice were secured. Three of the matings were wholly without issue. In no case was there more than one litter born, and 3 was the largest number of young obtained in a litter, the other two yielding only 1 and 2 mice respectively.

The second generation hybrids in their turn, yielded only 6 third-generation mice. The mating of these third-generation mice gave no offspring whatever and the strain thus died out in the third generation.

The individuals of the first hybrid generation from this cross were slightly smaller than the best Mus musculus derivatives, but larger than Japanese Waltzers. The mice in the second and third generations, however, were considerably smaller than the first generation hybrids and less vigorous by every criterion, viz., scantier coats, feebler pigmentation, less active, less prolific, and shorter lived.

The average age of the parent mice was 1 year 9 months. The average age of the first generation hybrids was 11 months 8 days. The average age of the second hybrid generation was 10 months. The average age of the third generation was only 5 months.

This strain, no. 479, is typical of every strain ever secured in this laboratory in crosses between Mus musculus or its derivatives, and Japanese Waltzers.

The type of mouse derived from these crosses undoubtedly explains the paucity of "takes" which Little and Tyzzer secured

after the first hybrid generation. An examination of their tables shows that in no case did they use any mice beyond the third hybrid generation and the diminution in their number of individuals from the first to the third generation is striking. It is evident that their results in these hybrid crosses were similar to those of this laboratory. It is to be expected that these feeble, short-lived strains would give a constantly diminishing number of "takes" as they give a constantly diminishing number and vigor of offspring. Therefore it is unnecessary to go far afield to find a type of inheritance to fit this number of "takes," particularly where such results conflict with the exact evidence of the inheritance behavior of spontaneous tumors. These authors were dealing with a feeble strain, which neither in offspring nor in tumor "takes" was vigorous like the parent strains. Whatever remains to be explained in their results would be furnished by the fact that in mass breeding the chance selections made for matings might pass by those mice which would have yielded progeny not immune to J. w. A.

To recapitulate then: where the hybrid strain is a feeble one like that resulting from the crossing of the Japanese Waltzer with Mus musculus and its derivatives, inbreeding lowers the progeny and the tumor production with more than normal rapidity, and speedily eliminates the strain.

There remains one point to be discussed in connection with the influence of inbreeding upon tumor production, viz., does inbreeding increase or does it decrease the ratio within a strain between the production of progeny and the incidence of tumor?

Lathrop and Loeb, in their paper quoted from above, state that in general the tumor rate is static within a strain, although in some few strains it shows a rise and in many cases it shows a fall.

Little and Tyzzer, on the contrary maintain that there is a steady decrease in the number of tumor "takes" of increasingly later generations, in their experiments, and deduce therefrom the application of a multiple-factor hypothesis for the inheritability of cancer. Their experiments, as stated above, were limited to three generations.

In considering this conflicting evidence, it must be remembered that Lathrop and Loeb are dealing with spontaneous tumors, while Little and Tyzzer are dealing with grafts. No further evidence of the intrinsic difference between spontaneous and grafted tumors is needed than Tyzzer's own results in later obtaining spontaneous tumors in individuals which had refused grafts (12).

Moreover, as stated above, Little and Tyzzer were obviously not demonstrating the inheritance behavior of "takes" of grafted tumor, but only the biological relation between tumor "takes" and a stock of mice of low grade metabolism and productivity. Their results were exactly what would be expected from such low grade, non-prolific stock.

A very large amount of evidence on the subject of the ratio between the production of tumor and of progeny has accumulated in this laboratory during the many years devoted to the study of spontaneous tumors. Obviously not all of this evidence can be brought within the confines of a single paper.

I have therefore gone over the strains already charted in Reports 5, 7 and 9 published from this laboratory, as these strains were selected with no reference whatever to the point here under discussion, and hence will yield typical and wholly unbiased evidence on the subject.

Chart 9 has been drawn up to show thirty-six of these strains which have already been published giving the number of the strain, the number of generations through which it was charted at the time of publication, the percentage of tumor production in the parent generation and in each succeeding filial generation, and the average rate of tumor production for the entire strain.

For example: Strain 246, charted through 5 generations, showed 50 per cent of tumor in the parent generation, 33 per cent in the first filial generation, 33 per cent in the second filial generation, 66 per cent in the third filial generation and 100 per cent in the fourth filial generation, or an average rate of 56.4 per cent for the entire 5 generations, etc.

Several points must be borne in mind in the study of this chart: (1) The matings made in these strains were all made *to test the Mendelian behavior of cancer;* not to find out how many

CHART 9

RATE OF TUMOR PRODUCTION
IN EACH GENERATION OF VARIOUS STRAINS

	STRAIN	NO. OF GENERATIONS	TUMOR RATE PARENT GEN.	TUMOR RATE F_1	TUMOR RATE F_2	TUMOR RATE F_3	TUMOR RATE F_4	TUMOR RATE F_5	TUMOR RATE F_6	TUMOR RATE F_7	AVERAGE RATE
1	246	5	50%	33%	33%	66%	100%				56.4%
2	245	4	100%	20%	60%	100%					70. %
3	215	5	50%	29%	18%	16%	33%				29 2%
4	65 Br_{IA}	6	50%	50%	50%	50%	0	0			33 33%
5	65 $^{Br}_{IB}$	5	50%	50%	50%	50%	100%				60 %
6	65 $^{Br}_{IIA}$	5	50%	50%	50%	50%	50%				50%
7	65 $^{Br}_{IIB}$	7	50%	50%	50%	50%	50%	100%	100%		64.28%
8	186 $^{Br}_{A}$	4	50%	100%	100%	100%					87 5%
9	186 $^{Br}_{B}$	5	50%	0	0	20%	0				14 %
10	202 $^{Br}_{A}$	5	50%	50%	50%	57%	50%				51.4%
11	202 $^{Br}_{B}$	6	50%	50%	50%	0	44%	100%			49%
12	112	4	50%	0	50%	50%					37.5%
13	124 $^{Br}_{I}$	3	50%	29%	25%						34.66%
14	124 $^{Br}_{II}$	3	0	50%	20%						23 33%
15	196	5	50%	60%	75%	0	100%				57. %
16	201	6	50%	50%	50%	50%	50%	100%			58 33%
17	405 $^{Br}_{B}$	4	50%	0	75%	50%					43.75%
18	104	5	0	0	50%	33%	100%				36 6%
19	384	3	50%	60%	100%						70 %
20	280	3	100%	100%	100%						100 %
21	139	3	100%	100%	100%						100%
22	281	5	100%	0	33%	50%	100%				57.6%
23	343	3	50%	57%	60%						55 66%
24	164 Br ABS	6	0	0	23%	40%	25%	100%			31 66%
25	450	3	50%	75%	100%						75 %
26	143	5	50%	50%	0	0	20%				24.%
27	413 $^{Br}_{A}$	5	0	40%	16%	57%	75%				37.6%
28	413 $^{Br}_{B}$	6	0	40%	20%	20%	16%	0			16.%
29	146 $^{Br}_{IB}$	8	50%	50%	50%	66%	100%	100%	100%	100%	77.%
30	146 $^{Br}_{IA}$	6	50%	50%	50%	25%	33%	50%			43.%
31	338 $^{Br}_{I}$	5	100%	50%	25%	77%	25%				55.4%
32	338 $^{Br}_{III}$	6	100%	0	38%	36%	75%	100%			59 16%
33	338 $^{Br}_{IX}$	4	100%	0	80%	50%					57.5%
34	338 $^{Br}_{XI}$	5	100%	50%	36%	50%	50%				57.2%
35	338 $^{Br}_{XII}$	5	100%	50%	0	50%	50%				50 %
36	338 $^{Br.A}_{XIII}$	5	100%	50%	0	100%	100%				70.%

tumors could be secured. (2) The matings therefore are of four kinds, double cancerous parentage; single cancerous parentage; parentage cancerous on one side, and heterozygous on the

other; and double heterozygous parentage (the latter tested out to show that individuals may inherit and transmit cancer although they themselves do not exhibit it, just as is the case with albinism). (3) The parent generation, then, in each of these cases shows 100 per cent of cancer, or 50 per cent of cancer, or 0 per cent of cancer, according to what test was being made. (4) As has repeatedly been published from this laboratory, cancer rarely develops in mice under 6 months old, frequently it does not develop until the mouse is 2 or 3 years old; so that the cancer ratio (except in the 100 per cent cases) is nearly always lower actually than it is potentially (*i.e.*, if more mice lived to a greater age more would exhibit cancer). Infections creep in and sweep the mice off in numbers sufficient to lower the cancer rate very considerably.

Examination of chart 9 shows little falling off of the cancer rate except in sporadic cases where infections crept in. On the contrary, the later generations show almost uniformly a tumor percentage higher than those of the earlier generations or than the average percentage for the strain.

Note the percentages shown in chart 10 of the strains under consideration, 83 per cent showed the same or a higher percentage of tumor in generations later than the parent generation, 88 per cent of the strains showed the same or a higher tumor rate later than the first hybrid generation, 93 per cent showed the same or an increased tumor rate later than the second hybrid generation, 79 per cent showed the same or an increased tumor rate later than the third hybrid generation, 88 per cent of the strains showed the same or an increased tumor rate later than the fourth hybrid generation, 100 per cent showed the same or an increased tumor rate later than the fifth hybrid generation, 100 per cent of the strains showed the same or an increased tumor rate after the sixth hybrid generation.

Chart 11 compares each succeeding generation with its immediate predecessor, giving the percentage of strains showing a steadily increasing tumor rate in each succeeding generation. Note the percentages: 58 per cent of the strains showed a higher

tumor rate in the first filial generation than in the parent generation. In 72 per cent of the strains the second filial

CHART 10

PERCENTAGES OF STRAINS SHOWING

RISE OR SAME TUMOR RATE AS PARENT GENERATION	83%
RISE OR SAME TUMOR RATE AFTER F_1	88%
RISE OR SAME TUMOR RATE AFTER F_2	93%
RISE OR SAME TUMOR RATE AFTER F_3	79%
RISE OR SAME TUMOR RATE AFTER F_4	88%
RISE OR SAME TUMOR RATE AFTER F_5	100%
RISE OR SAME TUMOR RATE AFTER F_6	100%

CHART 11

PERCENTAGES OF STRAINS SHOWING

F_1 HIGHER THAN PARENT GENERATION	58%
F_2 HIGHER THAN F_1	72%
F_3 HIGHER THAN F_2	71%
F_4 HIGHER THAN F_3	75%
F_5 HIGHER THAN F_4	88%
F_6 HIGHER THAN F_5	100%

generation showed a higher tumor rate than the first. In 71 per cent, the third hybrid generation exceeded the second in tumor production. In 75 per cent of the strains the fourth

hybrid generation showed a higher tumor rate than the third. In 88 per cent of the strains the fifth hybrid generation showed a higher tumor rate than the fourth. In 100 per cent of the strains the sixth hybrid generation showed a higher tumor rate than the fifth.

We have here then conclusive evidence that as the cancer ancestry behind a generation broadens and deepens, whether by the method of inbreeding or by the method of hybridization, the individuals of that generation tend to run more and more to cancer production. Let us refer again to chart 9 and note how many of these hybrid strains became 100 per cent cancer strains, which originated from 50 per cent cancer parentage (*i.e.*, one cancerous with one non-cancerous parent or one cancerous with one heterozygous parent) or 0 per cent cancerous parentage (*i.e.*, two heterozygotes) strains 246, 65 branch I A, 65 branch II B, 186 branch A, 202 branch B, 196, 201, 104, 384, 164, branches A, B and C, 450, 146 Branch I B.

Note also the cases where 0 per cent cancer strains eventuated from 50 per cent cancer hybrid crosses. These were some of the strains where the purpose was to show that the segregating out of cancer and non-cancer make it possible *speedily to eliminate all cancer from the strain.*

It is obvious that where scientists oppose the theory of the inheritability of cancer and reject the mass of indisputable evidence, it is because they do not wish to accept a theory which they deem unfortunate for the human race; note Ewing's quotation from Le Doux-Le Bard and his personal subscription thereto: "In the interests of the public this doctrine" (i.e., heredity) "ought to be combatted" (13).

If scientists subscribing to this idea would concentrate their attention upon the following facts of heredity, they would find encouragement not hopelessness, in the unquestionable fact of the inheritability of cancer:

(1) In hybrid crosses cancer and non-cancer tendencies *segregate out* and are transmitted as such.

(2) All human matings are hybridizations.

(3) Cancer behaves as a recessive.

(4) It can therefore be wholly eliminated by persistently mating individuals of cancer ancestry with individuals with no cancer in their ancestry.

(5) This elimination is infinitely better than any therapeutic procedure.

(6) The cure of cancerous individuals during the reproductive period, makes it possible for them to transmit cancer to a greater number of progeny (14).

(7) The insistence that cancer is not hereditary, and the continued matings of two cancer-bearing individuals, results in an ever increasing amount of cancer in the human race. If persisted in long enough, such a method will eliminate all the perfectly non-cancerous families.

The progressive increase of cancer in later generations of hybrid crosses demonstrated above, must not be attributed to inbreeding, as exactly the same progressive increase of cancer follows where every mating is a hybridization, if the cancer tendency is bred in constantly in each succeeding generation. In other words, it is *what is put into a mating, not the method of putting it in, that determines which characters shall appear in the offspring.*

CONCLUSIONS

1. Inbreeding is demonstrated not to be an influence in the increase or the incidence of cancer, but merely a method of analyzing a strain.

2. This method of inbreeding is of necessity used by every student of heredity, as there is no test of heredity which does not involve inbreeding.

3. Strains consistently inbred may produce 100 per cent, or 50 per cent or 0 per cent of cancer according to *how much cancer is bred in*, not in accordance with the method used.

4. The real effect of inbreeding is to eliminate cancer by eliminating the strain. It is hybridization which increases cancer by increasing the output of cancer progeny.

5. The ratio of tumor "takes" in increasingly later generations from hybrid crosses of low grade productivity, proves

nothing with reference to the inheritability of cancer, but demonstrates the biological relation between race vigor and the number of tumor "takes."

6. As the cancer ancestry behind a generation broadens and deepens the individuals of that generation tend to run more and more to cancer production. This is equally true of inbred and of hybrid generations, since the amount of cancer which comes out in the progeny depends upon the amount which is put into the ancestry, whether the method is inbreeding or hybridization.

7. It is therefore possible wholly to eliminate cancer from the race by not putting it in through the ancestry; this is true both in inbreeding and in hybridization.

8. In demonstrating the inheritability of cancer and of other tumor types in mice we have demonstrated their inheritability also for man and for every other species in which they occur, since cancer and non-cancer tendencies which *segregate out* in mice must segregate out also in every other species in which they occur, and this is the test of heredity.

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PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

TWELFTH ANNUAL MEETING

Held in Atlantic City, June 14, 1919

1. Report of the Council

The meeting of the Council was held at the Chalfonte Hotel, in Atlantic City, on the evening of Friday, June 13, 1919, i.e., the night preceding the day of the Twelfth Annual Meeting.

The following members were present: Dr. E. R. LeCount, president; Dr. William H. Woglom, secretary and treasurer; Dr. Harvey R. Gaylord, Dr. Francis C. Wood. Absent, Dr. H. Gideon Wells, Dr. James B. Murphy, and Dr. Robert B. Greenough.

The report of the treasurer included the sum of $125, $100 of which was given collectively by Dr. Harvey R. Gaylord, Dr. Benjamin F. Schreiner, Dr. B. T. Simpson, and Messrs. Millard C. Marsh and G. H. A. Clowes, and the secretary was requested to write to each of these gentlemen a note expressing the thanks of the Council for their generosity.

Means for increasing the circulation of the Journal were discussed and Dr. Gaylord suggested that Mr. Curtis E. Lakeman, secretary of the American Association for the Control of Cancer, be approached by Dr. Wood and asked to help in any way that he could to make the Journal known among members of the medical profession.

The names of three applicants came before the Council for election to the Association:

Dr. Ernest William Goodpasture, 7 Fremont Street, Reading, Massachusetts.

Dr. George H. Semken, 16 West 85th Street, New York.

Dr. Ross C. Whitman, University of Colorado, Boulder, Colorado.

Dr. Wood moved, and Dr. Gaylord seconded the motion, that these three applicants be elected to membership in the Association.

The resignation of the following member was accepted:

Dr. G. H. Mallett, 244 West 73d Street, New York.

Dr. Gaylord moved that Dr. Willy Meyer be elected councillor succeeding the retiring councillor. Dr. Wood seconded this motion.

The following officers were elected by the Council to serve for the ensuing year. Dr. H. Gideon Wells, president; Dr. Robert B. Greenough, vice-president; Dr. William H. Woglom, secretary and treasurer (re-elected).

The present Council, therefore, with the years of retirement, is as follows:

The Council continued in office the present Editorial Board which is composed as follows:

2. Lymphocytes and Cancer Immunity

Dr. Frederick Prime (New York):

SUMMARY

A series of experiments was undertaken in a strain of rats found to be resistant to repeated inoculations of the Flexner-Jobling rat carcinoma in 90 per cent of the animals, and in another strain of which but 15 per cent were resistant to the same tumor. The naturally immune rats were divided into two groups, one of which was given repeated exposures to x-ray, following the method advocated by Murphy, in amount sufficient to reduce greatly the lymphocytes in the circulating blood; the other group was kept as controls. Total white cell and differential blood counts were made before commencing the x-ray treatment, two days after the completion of the last exposure, and then at frequent intervals. Forty-eight hours after the completion of the last x-ray exposure all the groups of animals were inoculated with pieces of the Flexner-Jobling rat carcinoma, and the growths, if any, charted at weekly intervals thereafter. At the end of six weeks, out of forty-eight animals inoculated in each group those naturally immune animals whose lymphocytes had been reduced by x-ray treatment showed only 6.8 per cent of takes, whereas those which had not had any x-ray treatment showed 5 per cent of takes; a group of untreated controls, susceptible to the tumor, gave 90 per cent of takes. This experiment was repeated many times in both naturally immune rats and those proved immune through previous inoculations. In none of these was there any appreciable difference in the susceptibility of the animals to tumor inoculations, whether they had had the number of lymphocytes decreased by repeated exposures to x-ray or not. The same condition was found to be true also in rat sarcoma.

DISCUSSION

Dr. Francis C. Wood (New York): It seems to me that this paper rather effectively disposes of the postulated rôle of the lymphocyte as a carrier of immunity to cancer. I do not think it is possible to consider that the reduction of lymphocytes by the *x*-ray has any effect on the "taking" of tumors. What factors do play a part are unknown, but it seems to me that the lymphocyte as a carrier of cancer immunity disappears from now on.

Dr. W. C. MacCarty (Rochester, Minn.): In view of the fact that this paper is so contradictory to others, I would like to ask why this great variation has occurred.

Dr. Prime: I am afraid that the reasons for this variation have a great deal to do with the results that we get in all our work of this kind. Unfortunately a person working along one line in cancer research with too few controls gets one result and assumes a causal relation, and a second investigator who repeats his work on a larger scale gets a different result. It may be due to the tumor, or it may be due to the animal.

For example, the rats sold us by a certain dealer are almost or quite resistant to our rat tumors.

Dr. MacCarty: My reason for asking is this. I have a large series of cancer cases in human beings, and I am attempting to find out why some of them live so long. In patients where the glands are involved, earlier death might be expected. Yet some of them live eight or nine years, and I am at a loss to account for this.

3. The Relation of Pregnancy and Reproduction to Tumor Growth

Dr. Maud Slye (Chicago):

SUMMARY

In handling large numbers of mice with spontaneous tumors there is forced upon the observer from the very first the tremendous difference in the rate of tumor growth in non-reproducing and in reproducing females.

The same difference is noted in the non-reproductive and reproductive periods of the same female.

For this study thirty each of non-reproducing and of reproducing females with spontaneous tumors were selected. The tumors were all of the same type and of the same organ (with a few exceptions for purposes of comparison), viz., alveolar tubular carcinoma of the mammary gland.

Without exception the amount of tumor grown by a female while reproductive was much less than during her non-reproductive period.

Again, the amount of tumor grown by reproducing females was much less than that grown by non-reproducing females.

The normal course of these spontaneous tumors in mice that are not bred is very rapid, the mouse rarely living over six weeks and often less than a month after the appearance of her tumor. These tumors grow to a great size, frequently being as large as the body of the mouse.

However, when these tumorous mice are bred, the tumor scarcely grows at all during the reproductive period. The duration of the tumor is greatly prolonged, the mouse frequently living a year after the appearance of her tumor, during which time many of these mice bear from six to eight litters, aggregating from twenty to thirty-two young. When the mouse ceases reproducing, the tumor grows with tremendous rapidity and to great size, the female frequently living only six or eight days after the birth of the last litter. During this short period the tumor grows to many times its size at the date of the last litter.

In brief, during the six or eight days a tumorous mouse is non-reproductive, she grows much more tumor than during the eight months or a year that she is reproductive, the daily rate of tumor growth being far in excess of the daily rate during the reproductive period.

Two other factors must be taken into consideration, viz., (1) the age of the mouse, and (2) other complicating causes of death. Generally speaking, the younger mice show a higher daily rate of tumor growth than do the old mice.

Again, complicating factors, such as tapeworms or nematodes in the digestive tract, or any severe disease, greatly retard tumor growth.

But when these have been eliminated, two facts stand out with startling clarity and cannot be gainsaid: (1) Reproducing females grow much less tumor than do non-reproducing females of the same approximate age and general metabolic condition. (2) Reproducing females grow much less tumor while they are reproductive than while they are non-reproductive; in other words, when a mouse is producing embryos, she is not producing tumor in anything like the amount which she grows while non-reproductive. In striking contrast to these results is the relation between pregnancy and the infections common to mice.

If an infected mouse is bred, the infection is not held off for a year or more while she bears young; she is unable to produce any young at all and speedily dies of her infection. Or if a pregnant mouse contracts an infection, she rarely brings her young to birth.

The results of this study bring out with striking force the relation between tumor production and the production of young, showing them to be two related modes of growth.

DISCUSSION

Dr. Harvey R. Gaylord (Buffalo): I should like to ask Dr. Slye if she thinks it is possible to establish a strain of mice which will constantly produce a high percentage of tumors? I should like to inquire, also, into the frequency of tumors in virgin females as compared with animals which have been bred.

Dr. Slye: During the past ten years I have established hundreds of strains yielding a practically constant percentage of tumors. However, accidents which cause early death, epidemics of infection, lowering of the reproductive potency of a strain by insufficient diet or any other cause, or the running out of a strain by inbreeding, will of course lower the tumor percentage just as it will lower the reproductive percentage. I have shown before this Association strains showing 100 per cent of lung tumor or of mammary gland tumor, or 8 or 25 per cent of mammary gland tumor, or 60 per cent of liver tumor or ovarian tumor, etc.

The second question I am not prepared to answer at this time, as I have not yet compiled statistics on that point.

Dr. Gaylord: What I desire to determine is whether, in your opinion, a duly established strain, eliminating accidents, could be expected to continue to produce a definite percentage of tumors with such certainty that they could be used as the basis for experimentation. In other words, are the strains sufficiently constant in their percentage, after once established, to enable you to make deductions from specific experimental procedures?

Dr. Slye: I should not like to assume anything; in this work assumptions are dangerous. If you should take over a strain of my mice which was bearing 60 per cent of tumors in my laboratory, it might not go on producing 60 per cent in your laboratory.

Dr. Gaylord: If you have an established strain, I want to know about the stability.

Dr. Slye: There is no doubt about the stability of these strains, but they will run out, through inbreeding. As long as the strain persists, however, it will furnish tumors in a practically steady percentage, if it is correctly handled.

4. The Lymph-Nodes in Tumor-Bearing Mice

Dr. William H. Woglom and Dr. S. Itami (New York):

SUMMARY

This work is an extension of preliminary observations reported by one of us last year. At that time it was said that the lymph-nodes from rats with progressively growing or receding tumors exhibited no deviation from the normal, with the exception, perhaps, of some slight hyperplasia in the germinal centers.

The present series comprises a total of 213 animals—8 mice with spontaneous tumors, 129 mice with transplanted tumors, and 76 rats with transplanted tumors. The tumors were carcinoma, sarcoma, and carcinosarcoma.

It is not easy to establish a normal standard in these animals. Some nodes from supposedly healthy ones show a high degree of endothelial proliferation, others dilatation of the lymph-sinuses, still others a moderate hyperplasia of the germinal centers, and so on. On the whole, however, where a tumor has grown for five or six weeks an activity of the germinal centers in the lymph-node nearest the growth is sometimes found, and this activity is often somewhat more distinct than in mice without tumors.

The corresponding node on the opposite side is unaffected, whence it may be assumed that the activity is not due to a general systemic change. It is rather to be referred, perhaps, to some such local condition as the presence of a growing tumor, often slightly infected or containing necrotic material. This explanation seemed to us the more probable when we found, by injecting India ink, that the node studied in these experiments (the axillary node nearest the median line) is the one which drains the site at which the tumors were transplanted. Furthermore, nodes near receding tumors show a rather high degree of germinal activity. In any case the differences are slight, and it is not desired at this time to lay any particular emphasis upon them.

The number of nodes from spontaneous tumor mice is too small to serve as a basis for generalization; but it may be said tentatively that some of these nodes show a moderate degree of activity in the germinal centers and that others show little or none.

DISCUSSION

Dr. Wood: I think this illustrates very well what we know of human tumors of the breast, for example. We usually find such changes in human nodes more often in tumors which are either of the chronic mastitis type, where a great deal of absorption takes place from the ducts of material which is probably in many instances bacterially contaminated, or is irritating, as is shown by the cellular reactions which are produced around these ducts. Or we find it in carcinomata in which there is more or less necrosis of tumor cells.

Dr. E. R. Le Count (Chicago): What are the changes in the nodes? Are they in the germinal centers with overgrowth of lymphoid tissue, or are there changes in the sinuses as well?

Dr. Woglom: There are changes in the sinuses as well, but these occur also in mice that have no tumor. It is difficult to establish a normal standard. In mice apparently healthy there may be found an enormous dilatation of the lymph-channels, and half of the sec-

tion or more may be made up of dilated channels. Sometimes these are filled with desquamated endothelium. Sections from other nodes will be composed almost entirely of lymphoid tissue, with the sinuses and germinal centers hardly visible; in still others the germinal centers are rather distinct. We have found no changes in the sinuses of tumor-bearing mice that could not be duplicated in nodes from mice without tumors.

Dr. Le Count: Is it chiefly, then, a growth of the lymphoid tissue?

Dr. Woglom: Yes, sir. The cells are larger and clearer, and there are perhaps a few more mitotic figures. But then there is more chance of encountering mitotic figures because the area of the germinal center is somewhat larger. The change, however, is probably of no importance so far as tumor immunity is concerned.

5. (*a*) Concerning the Dosage of Rays

Dr. W. T. Bovie (Boston):

SUMMARY

If we look upon radiation as a flow of energy, we may indicate the dose by the total amount of energy absorbed, for it is a general law of photochemistry that the amount of photochemical change produced is, over a wide range of intensities, proportional to the total quantity of radiation absorbed. It easily follows that if the intensity of the radiation absorbed remains constant the amount of photochemical change is proportional to the duration of the exposure. But the intensity of the rays absorbed throughout an absorbing mass is not constant, for, due to spreading and absorption, the intensity decreases as the distance from the source increases, so that as the exposure proceeds equal increments of time do not result in equal increments of photochemical change.

It is impracticable to determine the relationship between the exposure and the amount of photochemical change by mathematical analysis. Using photographic paper, the author has mapped out equi-intensity surfaces about a linear source of radiation, such as a tube of radium emanation, both when the tube is embedded in a highly absorbing homogeneous medium, such as wax, and when only air is the absorbing medium. Intensity surfaces were also mapped out about tubes which were enclosed in the various applicators used in radiotherapy. From the shapes and dimensions of these surfaces, the influence of the volume of the radiated mass upon the amount of absorption was determined, and curves were exhibited showing the relation between the length of exposure and the total amount of photochemical change produced by the radiation.

5. (*b*) Concerning the Photo-Coagulation of Egg-White

Dr. W. T. Bovie:

SUMMARY

This is a preliminary report upon the influence of the hydrogen-ion concentration upon the coagulation by heat of a 10 per cent solution of egg-white which has been exposed to the ultra-violet radiation from a quartz mercury vapor arc. It was found that the greatest amount of coagulum is formed when the hydrogen-ion concentration of the solution is very close to its isoelectric point, and increasingly smaller amounts of coagulum are formed as the hydrogen-ion concentration is either increased or decreased. Similar non-radiated egg-white solutions showed no coagulum over the range of hydrogen-ion concentrations studied. The temperature of coagulation in these experiments was 18°C.

6. Incision of Tumors and Metastasis

Dr. F. C. Wood:

SUMMARY

In an experimental investigation of the effect of incising the Flexner-Jobling rat carcinoma upon the percentage of metastases, it was found that the incision of the tumor did not increase the percentage of metastases. It is, therefore, probable that the belief among surgeons that such incision necessarily hastens metastasis in human beings must undergo some revision.

DISCUSSION

Dr. MacCarty: I do not know how many times a year I have to decide the question of incision of tumors. I am very glad that these experiments have been done. I am glad also that Dr. Wood has been conservative, and speaks about *his* series of mice, because otherwise his conclusions might lead to indiscriminate practices. Whether or not tumors metastasize or grow more rapidly after incision does not make any difference in certain cases which I could mention. I am thinking of two or three instances illustrating this statement. One was a little child with a tumor on the sole of the foot. It had been treated by pastes, and a section had been removed by a surgeon and submitted to a reputable pathologist. He made the diagnosis of sarcoma and advised amputation. The case came to me for decision as to whether or not it was a sarcoma, and whether an amputation should be done. When I saw the section I decided that I would not make a diagnosis of sarcoma from the slide. The surgeon said "Now, what am I going to do?" and I said "I am going to incise the tumor, because if the child has a sarcoma it will never do the child any good to amputate. I shall not be satisfied until we examine the center of the tumor." So we did this, and I found a lipoma on the sole of the foot—quite a large lipoma,

distinctly encapsulated, but possessing a zone of inflammatory tissue which had been produced by paste, and there was an area probably 1 cm. deep with growing fibroblasts as a part of the chronic irritation; the first pathologist had received a section of that area. Incision distinctly settled the question and saved the foot of the child. Whether or not we believe in the incision of tumors, it is sometimes absolutely necessary to cut into a tumor; but we do not advise the incision of tumors as a rule. Another case in point was a man with a lesion of some sort on his tibia. He likewise had had a section removed, and this was sent to laboratories and diagnosed by three pathologists. They were reputable men, good, honest, well trained pathologists, who were not intimately interested in the patient. The sections were brought to me. I always try to look at things from the clinical standpoint, and study not only the sections, but the patient. I refused again to make a diagnosis. The men who had made the diagnosis had legitimate reasons for doing so, according to many traditions in pathology, but they knew nothing about the case. I saw the patient, got his history, and reported to the surgeon that I refused to make a diagnosis of sarcoma; the patient might possibly have a syphilitic condition. They said, "What are we going to do?" I said, "We are going to operate on him and take another piece out." As a matter of fact, I did not find out from the slide that he had syphilis, but I could not be positive of sarcoma. It was before the day when the Wassermann reaction was much in favor. I had said to the man, "You have either syphilis or sarcoma. If you have sarcoma, you are not going to be cured. If you have syphilis, you have a very good chance." We cut into the lesion, and the surgeon said he would not amputate it. The patient had syphilis, but had failed to answer in the affirmative. That is another example where it is absolutely necessary to cut into a tumor, whether or not you believe incision is a good thing. Almost daily the orthopedic surgeon comes in and says, "I have a bone tumor which I want you to see." I see the patient and the x-ray pictures, but how can I tell from these what is in the tumor? It is only fair to the patient to cut in and find out what is there. So far as my experience is concerned, I have not seen any bad results caused indubitably by the incision of tumors. I recall very vividly a man with an enormous sarcoma on his neck which was incised, and a week or two later it was nearly twice the original size; but whether this was due to the incision I cannot say. As Dr. Wood has emphasized, I hope incision will not become a general custom; the general surgeon should not get the idea that he can cut into every tumor. I have not seen a breast tumor incised for many years. As long as we can go around a tumor we ought to excise, and never incise it, and certainly never massage it.

Dr. H. H. Janeway (New York): I wish to express my gratification to Dr. Wood for doing this experiment, and to say that his results confirm our impressions at the General Memorial Hospital, where we

make biopsies as a routine. We have sent to us a great many inoperable tumors to be treated by radium, and we wish to have our diagnoses confirmed by microscopical sections. It has been our opinion that the incision is not accompanied by any diffusion of the growth. We have at the General Memorial Hospital a large number of cases strongly supporting this view, from which I will cite one example. A woman came to see us with a carcinoma of the tongue. A generous piece of the growth was removed two weeks before she applied to us for treatment. The section showed epidermoid carcinoma. This patient underwent a complete regression after one treatment by radium, and it is now two and a half years since this treatment was given, and she has developed no metastases to date. I give this as one instance of a large number of similar cases which impress us strongly that incision of a tumor for diagnosis is not harmful. I agree with Dr. MacCarty that much care in taking specimens should be exercised. In the majority of tumors of the breast we prefer not to make biopsies. In cases which are to be operated on there is no need of them.

Dr. Lockrey (Philadelphia): I had a tumor on my own tongue which was diagnosed as an epithelioma. There was a piece taken out, and it was found to be squamous-cell epithelioma. I had radium used, as well as *x*-rays, and an electric cautery. Three years have elapsed, and there has been no recurrence.

Dr. LeCount: It does seem as though it were necessary for someone to bring forward evidence to demonstrate that the opposite is true; in other words, the burden of the proof is with those who wish to demonstrate that incision is harmful.

7. Basal-Cell Epithelioma of the Rat

Dr. Dudley H. Morris (New York):

SUMMARY

This tumor was a very slowly growing variety, not continuously transplantable, but it grew for a long time when re-implanted in its original host. In other words, the tumor not only in its biological qualities, but also in its morphology, resembled very closely the basal-cell tumors of the skin in man.

8. Effect of Inoculation Technic upon Percentage of Tumor Takes

Dr. E. G. Cary (New York):

SUMMARY

The usual procedure is to wet the skin with 95 per cent alcohol and to insert a large caliber platinum needle carrying the graft under the skin of the abdomen, pushing it into the axilla where the tumor par-

ticle is deposited by a trochar. The method has been criticised as leading to bacterial infection or the tumor, but a series of experiments was tried where the skin was prepared in various ways, by epilation, shaving, and disinfection, especially with iodine. It was found that no greater percentage of takes could be obtained by such refinements, and that the tumors growing by the ordinary method of implantation are no more apt to contain bacteria than those implanted with the most painstaking surgical technic. It is therefore unnecessary to employ in tumor transplantation any especial means of disinfection of the skin of the animal to be grafted.

9. Arsphenamine and Tumor Growth

Dr. F. C. Wood:

SUMMARY

There is no reason to think that the administration of arsphenamine will stimulate tumor growth, and it is well known that neither it nor compounds of arsenic cause tumors to regress. It is always difficult to judge of the rate of tumor growth in man, even when untreated, and as there have been several instances where patients or their families felt that tumors were stimulated by the giving of arsphenamine, and had threatened legal proceedings, it seemed worth while to conduct a small series of experiments to note the effect of the administration of the drug. A series of mice was inoculated with a tumor, the growth rate of which was known, and the developing neoplasms were carefully measured at regular intervals. Not the slightest difference in growth rate could be detected by careful measurements between those animals receiving none of the drug and those which received varying dosages. It may be concluded, therefore, that arsphenamine neither stimulates nor inhibits tumor growth.

10. Attempt to Influence Tumor Growth

Dr. F. C. Wood:

SUMMARY

Several years ago Benedict stated that the administration of phlorhizin caused the disappearance of the Buffalo rat sarcoma, even when the tumors had grown to large size. His work was repeated by Wood and McLean with negative results. Benedict replied that the reason for this failure to confirm his results was that an insufficient quantity of phlorhizin had been given, and explained that he had used large rats and had periodically suspended the administration of the drug to enable the animals to recover from the toxic effects. The work was therefore repeated by Wood, following Benedict's technic, with negative results, but the findings were not regarded as of sufficient general importance to warrant publication until it was called to our attention

that Ewing, in his recent book on neoplasms, has cited Benedict's work as valid. It seems desirable, therefore, to record the fact that these experiments were repeated with phlorhizin from three different makers, and on large animals, the doses being spaced as was done by Benedict. Not the slightest effect was noted on the Buffalo rat sarcoma. It is evident that Benedict's results were due entirely to spontaneous disappearance of the tumor and not to any therapeutic effect of the phlorhizin, thus confirming the published conclusions of Wood and McLean.

DISCUSSION

Dr. Bovie: This discrepancy in results reminds me of some blood counts. The clinician had drawn a very nice curve showing the results of radium treatments. It was a thin line, an average of several curves. I suggested that he represent the averages by a line wide enough to include a 50 per cent error in the data. This was some six months ago, and so far as I know the data have not been published. The width of the average curve drawn to include one-half of the data was so great that the curve was practically meaningless. A statement of only the averages of experimental data may be very misleading.

Dr. Wood: Dr. Prime and myself have spent many weary hours in attempting to compute the errors in lymphocyte counts. I had planned to use a similar wide strip showing the error inherent in the enumeration methods as given in Ross' error tables. We came to the conclusion that no two mice ever have the same blood count even approximately, and that there is no use in trying to make an average.

11. Pathological Changes Accompanying Injections of an Active Deposit of Radium Emanation

Mr. Halsey J. Bagg (New York):

SUMMARY

Following injections of an "active deposit" of radium emanation, by either the intravenous or subcutaneous method, there is a diffusion of the radio-active substance throughout the animal body, which subsequently results in pathological changes in the various organs. These changes were found to occur in the liver, lungs, kidneys, adrenals, spleen, bone marrow, brain, and the vascular system. The most interesting changes were those found in the liver, resulting from comparatively small doses of radium injected subcutaneously. A fatty degeneration, associated with many giant cells and hyperchromatic nuclei, was found in the liver for a comparatively long time after the treatment.

Following large doses of radium, congestion and hemorrhages were frequently found in practically all the organs, and in the severe, acute cases the animals died after showing symptoms of marked enteritis.

The most frequent pathological condition in the kidney was a granular degeneration and erosion of the renal cells. In the bone marrow there was a destruction of the cells, resulting in subsequent replacement by blood. Congestion of the spleen was the most constant feature following radium treatment, and in some cases this was associated with hemorrhages and the destruction of red blood cells.

The method of injection appears to determine, to a certain extent, the severity of reaction in certain organs. For example, following subcutaneous injections, there were no appreciable lung lesions, but with intravenous doses of about the same strength, proliferation and desquamation of the epithelial cells of the bronchi, marked edema, congestion, and hemorrhage were found to occur.

A similarity was noted in the tissue reactions when radium was injected intravenously or subcutaneously, and when it was applied externally.

Concerning the fate of radium after its injection into the animal organism, it was found that the liver, gastrointestinal tract, kidneys, lungs, and spleen received the greatest amount of radio-activity.

DISCUSSION

Dr. James Ewing (New York): Of course it is obvious that the object of this work is to extend the therapeutic action of radium from a local agent to a constitutional one, for we have to admit that up to date radium is not this sort of a therapeutic agent. I am very much interested in the results Dr. Bagg has obtained, especially in the ones which would lead to caution in the use of intravenous injections. In animals that are in a fair state of health sixty or seventy days after injection we find in the liver a very pronounced, and in my experience, specific lesion. Of course I use the word "specific" with every caution and reserve. I have never seen anything like it—huge hyperchromatic nuclei, extensive changes in the cytoplasm, and a lesion which in my experience is specific. The fact that relatively small amounts of the agent can produce a lesion which develops or persists after sixty or seventy days have elapsed would certainly suggest caution in employing this agent in human beings, because we do not know what is going to happen a year after the injection. One of the conclusions that I have drawn from this work is that one should count on a period of observation of not less than six or twelve months before estimating the effect of radium deposit on the patient. It is quite obvious from Dr. Bagg's work and from the work of others before him, that it is possible to obtain a constitutional effect, and a very pronounced effect, from injections of radium deposit. Just what it will lead to in the treatment of disease is hard to say. The lymphomatous group suggests tself as the first type of disease in which to use it. Back in 1910 in Paris, I was told by a French observer that he had seen the disappearance of a lymphosarcoma in a child after the injection of radium salts. On the basis of this work Dr. Janeway has already carried out some clinical observations on the effect of radium deposit injected intravenously.

Dr. MacCarthy: I should like to ask whether the clinicians who have been using radium have noted any degenerative changes in the rest of the organs, and whether the action of radium is transferred to other parts of the body from a local point.

Dr. LeCount: I should like to ask if the spleen is enlarged, and also whether these rather interesting changes in the liver can be explained.

Dr. Bagg: In regard to the first question, I do not know whether any scientific work has been done. I believe the General Memorial Hospital is one of the first to employ the method. Perhaps Dr. Janeway can answer your question.

Dr. Janeway: Our experience in the injections of active deposits has been limited to two cases. Unfortunately peculiar circumstances made it impossible for us to carry out the examinations necessary to answer Dr. MacCarty's question. The most we were permitted to do was to make blood counts at intervals on these patients. I wish to emphasize again the need for caution in drawing conclusions of which Dr. Ewing has spoken. The first patient received an injection in October. He left the hospital and I traced him with considerable difficulty. The second patient left, the hospital after the injection, and we were able to trace her also with considerable difficulty. I had a second blood count made in her case at the hospital about two weeks ago. I do not think I can make any statement regarding the manifestation of the toxic changes such as Dr. Bagg speaks of from the result of the clinical examination of these patients. There is no doubt that the injection does produce very severe toxic effects. The second patient I treated received rather a large dose. The reaction after it demonstrated what constituted a maximum or even an over-dose. She was prostrated for at least three weeks' time, and for five days after the injection she vomited continuously. However, as far as can be told to-day, the end result is encouraging. She received the injection in February. At that time she had a white blood count of 30,000, with 100 per cent of lymphocytes. When I last saw her the blood count was 10,000, with practically a normal distribution in the differential count. She recovered after a month's time from her period of prostration but, as Dr. Ewing has said, this does not mean that there may not still be severe changes in the internal organs. However, she now feels better than for a long time before receiving treatment.

Dr. Bagg: In regard to the question of tissue changes, I do not think we can formulate any definite theory, but it does seem possible that this very active substance, coming in contact with the cells, may change the permeability of their membranes. It looks as though a considerable amount of water had been taken up by the cells, and as though following that phenomenon there had occurred a granular and fatty degeneration. It is quite possible that this change may have resulted in the metabolism of the cells being disturbed, and that being the case, a subsequent degeneration might be expected.

Dr. Le Count: Is the spleen large?

Dr. Bagg: Usually it is somewhat reduced in size. I think that there has been a certain amount of clinical observation showing like results following similar methods.

12. (*a*) Fluctuations in Induced Immunity

(*b*) Fluctuations in Concomitant Immunity

Dr. F. D. Bullock and Dr. G. L. Rohdenburg (New York): Read by Dr. Rohdenburg.

SUMMARY

(*a*) The authors call attention to the fluctuations in the percentage of induced immunity observed in various experiments. The importance of the recognition of these variations is emphasized, and evidence is produced which apparently proves that they are due to the neglect to use pure bred animals as hosts. When pure strains are employed as recipients of the tumors, the variations are negligible.

(*b*) Attention is called to the fact that the behavior of a tumor as an immunizing agent is not constant. From the various experiments recorded it appears that the variations are due to changes in the tumor and not to changes in the host. It was shown in the previous paper that analogous variations in induced immunity were due to variations in the strain of animals used.

DISCUSSION

Dr. Woglom: There is one aspect of Dr. Rohdenburg's work which might have been attacked if we had not been careful to control it. I refer to the personal equation in interpreting the results, a factor whlch always has to be taken into account. I took his charts and, without seeing his curves, constructed curves of my own from them; it was remarkable to see how the two series coincided.

Dr. Wood: Another point that leads to impartiality in the Crocker Laboratory is the fact that the tumors are all charted by a laboratory helper who knows nothing of the experiment. No investigator charts the tumors with which he is working, so that the personal element in determining the size of the tumor is eliminated.

Dr. Ewing: I should like to inquire what the significance of all this is regarding the nature of what you call immunity.

Dr. Gaylord: It seems quite logical that Drs. Bullock and Rohdenburg should have finally covered this most interesting phase of cancer research by careful study. The conflicting reports of various experiments in attempting to repeat work of a positive character previously published, has convinced all of us in the past that very striking fluctua-

tions in the conditions surrounding these experiments really existed. I recall that the Buffalo rat sarcoma at one time gave alternate high and low percentages of successive inoculations in each alternate generation over a considerable period, and in its relation to immunity von Dungern has already emphasized such fluctuations. In the early days of experimental cancer research, positive findings were controverted by negative results in the hands of other experimenters, and in many cases these questions became controversial. This is an extremely interesting and satisfactory paper and should definitely clear this field. It will serve also to re-establish much positive work which has frequently been apparently disproved, and it will greatly strengthen the foundation of experimental research in tumors and immunity in animals.

Dr. Isaac Levin (New York): The real difficulty in all our research in cancer immunity lies in the fact that the cancer cell has peculiar characteristics, and does not behave in an identical manner at different times. This is true in animal as well as in human cancer. A tumor may lie quiescent for several years and then, without the action of any extrinsic agent, become more or less malignant. It is quite possible that the different results often obtained by several investigators with the same tumor and the same method of immunization, may be due to the variability of the tumor employed.

Dr. Le Count: Has anyone any observations bearing on fluctuations and their behavior in a standard tumor stock which has been used for many years?

Dr. Levin: I have been working with the Flexner-Jobling tumor for several years, and I can testify that it has behaved differently as regards its transmissibility during that time.

Dr. Le Count: Is this the experience of others working with the same stock?

Dr. Gaylord: There are many instances of that kind. In our first experiments in immunity, a controversy arose in regard to our results; and though we tried several times to repeat them, we could not. After two or three years we were able to do so.

Dr. Rohdenburg: In answer to Dr. Ewing's question, I shall have to admit that these experiments do not show very much. Originally we were trying to solve a problem which came up several years ago: the problem of what particular factors are at work in the production of immunity in animals. Varying results were being reported, and the question arose, who was right. As it turned out, any of them may, perhaps, be right for a given tumor at a given time. I think Dr. Gaylord is correct in saying that, as a result of this demonstration, we may be able to correlate many things that at present are rather at variance.

13. Malignancy of the Crown Gall and Its Analogy to Animal Cancer

Drs. Isaac Levin and Michael Levine (New York). Read by Dr. Levin.

SUMMARY

Previous investigations of the writers have indicated that the crown gall presents an ideal material for the cellular study of the cancer problem. It appeared imperative, therefore, to estimate the true relationship between the crown gall and animal cancer, and this was the object of the present investigation. A large number and a great variety of plants were inoculated by Dr. Smith's method. The research shows that certain of these plant-tumors behave, both morphologically and biologically, as benign growths; while others, inoculated in the same manner, appear to be true malignant tumors. Certain phases of the development of these tumors resemble granulomata as near as an organism void of blood or lymph circulation may do so. In accordance with the findings of Dr. Smith a number of crown galls were obtained containing leafy shoots.

The microscopical study of the material revealed conditions which differ materially from the conditions obtained in animal cancer. Usually the entire gall presents a uniform morphological appearance of small, young, undifferentiated cells. In other galls the central growing part presents the usual appearance of a crown gall, while the periphery shows the development of adult differentiated tissue (parenchyma), rudimentary organs (conducting system), or even a whole rudimentary organism (leafy shoot).

The conclusion to be derived from this study is that a rapidly developing simple crown gall presents considerable analogy to animal cancer, and offers an ideal material for the cellular study of the latter condition. On the other hand, the structure of the growing central part of the crown gall does not change with the structure of tissue inoculated. It represents, therefore, only one form in the large group of pathological growths designated under the name of cancer. Until it can be demonstrated that the same microorganism may produce a crown gall with an entirely different morphological structure derived from a selected tissue it cannot be asserted on the basis of this study that one parasite must be the cause of all human cancer.

DISCUSSION

Dr. LeCount: I am sure that you all remember the interest aroused several years ago by the work of Dr. Smith; and perhaps you have been often asked, as I have been, whether proof had not at last been brought forward that cancer is a parasitic disease. I think this paper should be fully discussed.

Dr. Gaylord: I want to ask Dr. Levin if he does not consider that the question of tissue differentiation in crown gall is not well covered by the marked differentiation found in the transplanted osteochondrosarcoma of chickens. This tumor, one of Rous's caused by a filterable agent, is a tumor composed of cartilage and bone, portions of which cease growing and lose their neoplastic characteristics, leaving spheres of fully developed bone containing red bone marrow. Dr. Levin says that the peripheral cells at the margins of one of the crown galls, of which he shows us a lantern slide, have lost the tumor character and present the appearance of normal plant cells. I should like to ask whether he considers that in these cells the process of crown gall has terminated, leaving differentiated cells behind. If he makes that interpretation, then the conditions are exactly analogous to those in the osteochondrosarcoma of chickens, and his contention that these conditions in crown gall are different from anything found in animal pathology is not well grounded. The question of the analogy of crown gall to tumors in animals was emphasized by Jensen in 1910, when, at the meeting of the International Cancer Congress in Paris, he discussed these plant tumors, in showing some very interesting specimens of crown galls implanted from white beets into red ones. He stated that the analogy between the plant tumors and those of mice, with regard to transplantation, is extremely close; and after citing many points of similarity, said, furthermore, that the crown gall is, in his opinion, a true neoplasm because no etiological organism had ever been demonstrated. Curiously enough, at that time Dr. Smith had already demonstrated his *Bacillus tumorfaciens*. I should like to ask Dr. Levin if, in his opinion, there is any question of *B. tumorfaciens* being the sole cause of crown gall. Dr. Smith's interpretation of the significance of crown gall is just the reverse of Jensen's; Jensen regarded crown gall as a neoplasm because no organism had then been found in it, while Dr. Smith maintains that because this plant tumor is caused by a bacillus, its etiology affords strong evidence of the probable parasitic nature of neoplasms in animals and man. I have always felt that there would have to be some discount of any sweeping conclusions of this kind; the great variety of tumor formation in man, precludes in itself the possibility of a single causative agent.

Dr. Georgine Luden (Rochester, Minn.): I want to ask Dr. Levin whether he has tried to reproduce some experiments which were done last year or the beginning of the previous year by Dr. Smith, as a result of which he rather discounted the significance of his own bacillus, because he was able to get the same kind of tumor in plants by using dilute alkali, dilute acid, acetone, and various kinds of oils. He obtained the same results merely by exposing his plants to chemical vapors, and came to the conclusion that, as he was able to produce these same types of reaction by chemicals, the bacillus is not at the bottom of the proliferation, but that this is due to a chemical reaction in the plant, the chemicals contained in the bacillus, or in the plant itself being responsible for the formation of the tumors.

Dr. MacCarty: For a number of years I have been very much interested in Dr. Smith's work, both in his own laboratory and in my own, and within the last two months I have spent some time in his laboratory going over his material, so I know something about his work and the historical development of it. I think our speaker has been a little too rigid in his criticism; in my own opinion this work has been one of the most important pieces of biological research of the last twenty years, even though Dr. Smith may have made some mistakes in applying his results to animal pathology. If such occur, they do not seem to me to be very serious. One illustration which Dr. Levin uses I should like to question; his statement, namely, that tuberculosis in a node may become either Hodgkin's disease or lymphosarcoma. I should like to know if there is any scientific evidence that a tuberculous node can ever become either lymphosarcoma or Hodgkin's disease. In regard to differentiation in the cells of human neoplasms, I know of no case where cells have been completely differentiated. If they had been, they would have produced an accessory tissue or organ, not a tumor. In these tumors of Dr. Smith's, differentiation is incomplete, as it is in human neoplasms. Those tissues of the human body which are most highly differentiated in malignant neoplastic conditions are the cells of rectal carcinoma and of squamous-cell epitheliomata. But I have never seen any malignant neoplastic cell that could be called completely differentiated, after examination with a high power lens. The nucleus is not differentiated. I believe pathologists have been a little dogmatic in putting granulomata into one group and new growths in another; it seems to me that we are dealing with the same biological process of defensive reaction to environment, and that these two conditions are but phases in the biological reaction. Environment certainly varies in these cells. In neoplasms the cells tend to differentiate if the environment is favorable, but, as I said, I have never seen one completely differentiated. Instead of attempting to separate regeneration and neoplasia sharply from one another, we should take into consideration the question of texto- and cyto-differentiation and regeneration, which are most important factors both in regeneration and in neoplasia. I do want to emphasize my belief that when the essayist criticizes Dr. Smith's work and conclusions, he should consider the evolution of his studies. I have spent hours trying to convince Dr. Smith that these crown galls are not due to an organism. As a matter of fact, he is now producing neoplastic conditions by purely physical means; he can initiate them in certain begonias by merely transplanting from one pot to another, in other words, by an interference with constitutional conditions plus a local destruction. I am looking at these things from the purely biological standpoint, in dealing with the regeneration of cells. The nearer we come to looking at the question of neoplasia from the standpoint of regeneration, the nearer we shall come to the real biological facts relative to neoplasia.

Dr. Woglom: I should like to ask if these so-called embryomata arise only at the site of accessory buds; if so, may their importance not have been overestimated, since no unusual stimulus is required to complete the development of accessory buds? Again, may I ask whether the so called embryomata contain representatives of root, stem, and leaf? In order for these lesions to merit the term "embryomata," should not some of them at least have all three of these parts?

Dr. LeCount: It is very interesting to have applications made to animal pathology by a botanist, and to have similar experiments made by a worker in animal pathology, because only in this way are we going to ascertain the truth. In the lantern slide where there was crown gall in the center and a scar at the periphery, are the cells about the periphery crown gall cells? In regard to the osteosarcoma referred to by Dr. Gaylord, I once studied a malignant tumor arising in the upper end of the tibia[1] in which the tumor cells did nothing but make bone. They did not make cartilage at all. In this the growth differed from all the osteosarcomas I have seen. There were metastatic growths in the lungs, and upon arriving at the pleura the tumor cells had made bone there, which finally reached such a stage of maturity that no one could doubt the differentiation. One could not have distinguished that bone from bone altered a little by disease, or even from normal bone, because it had differentiated into complete Haversian systems, with lamellæ, bone marrow, depots of red blood cell genesis, and fat.

Dr. Levin: I fully expected that the discussion of my presentation would embrace the whole field of pathology and biology. Dr. Gaylord apparently still has some sympathies left for the parasitic theory of cancer. Chondroosteosarcomata and osteomata are tumors which consist of bone-forming cells, and are not comparable to either embryomata in man or to the crown gall, which forms leafy shoots. The latter are complete rudimentary organisms and are not comparable to anything observed in animal tumors. Now to take up the statement of Dr. MacCarty that I am criticising Dr. Smith's work, or undervaluing it; I am indeed far from it. I think he has done a wonderful piece of biological research, and I believe that the crown gall is the most ideal material for the study of biological phenomena of cell proliferation. I have no argument with the investigations of Dr. Smith, only with some of his conclusions. When an investigator in botany makes sweeping deductions in the field of human cancer, the latter urgently need revision. Our thesis is the "*Malignancy* of the crown gall and its relation to animal cancer," not the general investigation of crown gall. In answer to Dr. Luden: we carried out experiments with chemicals also, but what we obtained was not a true crown gall, and surely not a malignant tumor. All irritants will readily produce a wart-like

[1] Johns Hopkins Hospital Bulletin, 1909, xx, 361–370.

structure, a benign growth, on a plant. Our investigations, which will be reported later, seem also to indicate that microorganisms other than the *B. tumorfaciens* may produce conditions analogous to a true crown gall. Dr. MacCarty doubts whether tuberculous nodes may change into Hodgkin's disease or into a lymphosarcoma; yet there is very authoritative opinion which claims that Hodgkin's disease is always due to the tubercle bacillus. But one thing is absolutely certain, and that is that Hodgkin's disease and lymphosarcoma always develop on the basis of a tuberculous or other infectious granuloma. I do not believe that Dr. MacCarty quite followed me in my remarks on cell differentiation. I maintain that tumors in the animal organism consist of undifferentiated tissue. I stated that crown gall is also an undifferentiated tissue, and thus far is analogous to animal tumors, but just when it ceases to be undifferentiated and develops into differentiated tissue with specialized function, then its similarity to animal tumors ceases. Dr. Woglom is correct; an embryoma usually occurs where accessory buds take place, and the buds may form as easily without the tumor. The leafy shoots always develop from the ordinary crown gall cells. In answer to Dr. LeCount's remarks, I can say that the adult differentiated stem cells observed at the periphery of the crown gall are newly formed from the crown gall cells. Again it must be stated that the leafy shoots developing at the periphery of the crown gall are not analogous to osteomata any more than to embryomata. Dr. Smith has made the mistake of overvaluing something, because he has not clearly understood the significance of human embryoma, of teratoma, and the subsequent development of a malignant tumor within the latter tumors. The leafy shoots or other specialized tissue develop subsequently to and upon a presumably malignant tumor, and therein lies the main difference between the animal and plant tumors.

Dr. LeCount: If you had seen the peripheral tissue alone would you have known that it was crown gall?

Dr. Levin: No.

Dr. MacCarty: In regard to calling these embryomata and teratomata, I might state that when Dr. Smith had made his experiments he sent his results to me, and I went to Washington to keep him from calling them embryoma and teratoma. I am not in favor of calling such neoplasmata, either in plants or animals, by such names.

Dr. Gaylord: I think Dr. Levin misunderstood me entirely. I did not mean to infer that he was criticizing Dr. Smith. I have never sympathized with the idea that crown galls produce teratoid tumors. I have much the same opinion that Dr. Levin has. In regard to the remarks that have been made about inducing cell proliferation in plants with chemical and other means, that is not remarkable. The

astonishing thing was the discovery that a bacterium produced a pseudo-tumor. I think that no one has any idea today that one organism is the cause of cancer, no matter to what extent they may believe that certain tumors are parasitic. But I think that everyone can see that cancer is a great group of diseases. I should take issue with Dr. Levin's remark about the origin of the osteochondrosarcoma of chickens. These tumors arise from undifferentiated connective tissue, the same undifferentiated tissue from which Rous has been able with two other viruses, one derived from a spindle-cell sarcoma and the other from a spindle-cell sarcoma which developed extensive clefts lined with endothelium, to produce tumors true to these types by injecting with these viruses. In this instance it appears that the virus of osteochondrosarcoma acts in such a way upon these cells as to cause them to form cartilage and bone.

Dr. Levin: Dr. Gaylord started by saying that he saw differentiation of tissues in a malignant tumor, and then changed it to a "somewhat" malignant tumor. No truly malignant tumor will behave in this manner. Moreover, as stated above, a bacterium easily incites cell proliferation in a plant, since this is the only way in which a plant may react to irritation. The *Plasmodiophora brassicae* causes a proliferation not only in the cells which contain the parasite, but also in the free cells in the neighborhood.

Dr. LeCount: Your Chairman feels that there is no disagreement needing further discussion.

14. (*a*) Tissue Transplantation and the Lymphocytic Reaction

Dr. Leo Loeb (St. Louis):

SUMMARY

In the case of homoiotransplantation of various tissues a lymphocytic reaction appears around the transplant, which is absent in the case of autotransplantation.

This reaction is a graded one, and indicates the relationship between donor and host. In case of transplantation into near relatives (from brother to brother) the reaction appears later than in the case of transplantation into individuals who are not related to each other. The degree of strangeness of the substances produced by different members of the same species varies in accordance with the degree of relationship between host and donor, and the lymphocytes sense these differences. The reaction of lymphocytes which is elicited through homoio- or syngenesioplastic transplantation is a primary reaction of the lymphocytes and not a reaction caused by immune substances.

The fibroblasts and capillary vessels are also specifically influenced by syngenesiotoxins and homoiotoxins. In the case of homoiotrans-

plantation fibroblasts are attracted strongly by the transplant, while capillaries are attracted only weakly. The reverse condition holds good in autotransplantation. In syngenesiotransplantation the conditions are somewhat intermediate, but on the whole, especially so far as practical results are concerned, syngenesiotransplants approach more closely homoio- than auto-transplants. The reactions called forth through immunization against homoio-tumors resemble closely those found in the case of homoiotransplantation of normal tissues.

(*b*) The Invasion of Blood Vessels by a Normal Tissue Transplant

Dr. Loeb:

SUMMARY

Regenerating tissue may show many characteristics of cancer tissue. We have recently had an opportunity to observe even the penetration of newly formed capillaries by regenerating strands of thyroid tissue. From here the invading tissue was carried to arteries, and within the lumen of the latter relatively large quantities of this tissue became fixed to the wall of the vessel and sheathed in by endothelium. The invasion of capillaries was not confined to one place, but occurred in several vessels simultaneously. The invading tissue not only remained alive in the vessel wall, but continued to proliferate mitotically.

This observation was made in a case of autotransplantation, seven days following the implantation.

While regenerating tissue may in certain important aspects closely resemble cancer tissue, still it would be false to conclude that cancer tissue is merely normal regenerating tissue which continued to proliferate because it did not make proper connections with its own kind of tissue.

LYMPHOCYTES AND CANCER IMMUNITY

FREDERICK PRIME

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

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Since, following the suggestions of Da Fano and others, Murphy (1) and later Murphy and Morton (2) have laid great stress upon the rôle played by the lymphocyte in the production of immunity, much work has been done with widely varying results. Sittenfield (3), for example, found that neither increase nor reduction of the lymphoid elements in the blood had any influence upon resistance or susceptibility to tumor growth, when the Flexner-Jobling rat carcinoma was used. With such differences in the results of published experiments it seemed advisable to continue investigations of this problem of tumor biology in order to correlate, if possible, the work already done. This experiment is one of a series undertaken with this end in view.

Recently the Crocker Fund obtained a strain of rats which was found to be resistant in about 90 per cent to repeated inoculations of the Flexner-Jobling rat carcinoma, the inoculated tumors receding after about fourteen days. Another available strain of rats, on the other hand, showed but about 15 per cent resistance to the same tumor. These naturally resistant rats, together with others of the same strain already proved immune to the Flexner-Jobling rat carcinoma by previous inoculations, were divided into two groups, one of which was given sufficient repeated exposures of x-ray greatly to reduce the lymphocytes in the circulating blood, while the other was kept as controls. Both total white and differential blood counts were made before commencing the x-ray treatment, two days after the completion of the last exposure, and subsequently at frequent intervals. In all

cases the blood was taken from the animals under similar conditions. During the experiment all the animals remained under the same conditions as regards both feeding and treatment.

For five consecutive days the rats were given exposures of unfiltered x-ray from a Coolidge tube run from a Kelley-Koett

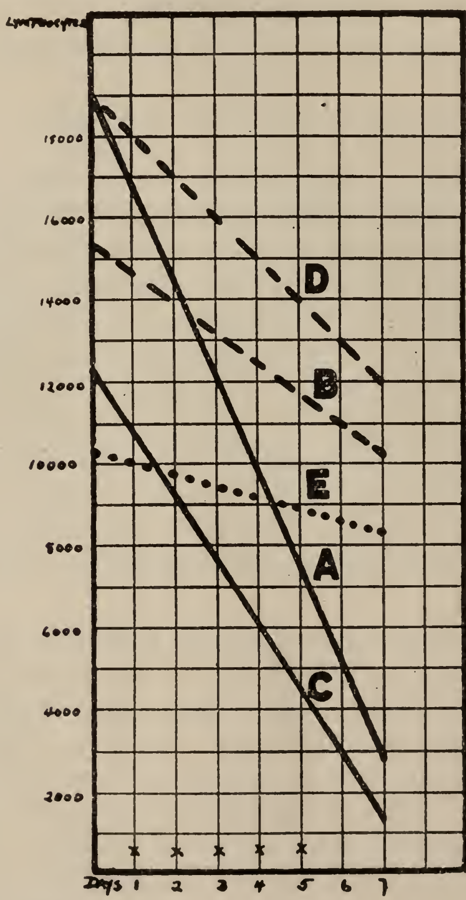

Fig. 1

rectifying transformer. The distance from the anticathode to the back of the animals was 30 cm., spark gap 3.5 inches between points, milliamperes 10, exposure four minutes, a total of 10 Holzknecht units being given. The pastilles were checked on

both a Holzknecht and a Cox radiometer, the readings being very consistent. This is a smaller dose than that given by Murphy, but we found that when we used a total of 30 Holzknecht units, as he reports, our animals did not live long enough to complete our experiments. Text figure 1 shows the composite blood counts before, and forty-eight hours after the completion of the x-ray treatments. The blood counts of the control groups for the same periods also are shown on the same chart. Each line represents the composite lymphocyte count per cubic millimeter of blood of each group of rats.

Group A. Forty-eight resistant rats given x-ray.

Group B. Forty-eight resistant rats kept as controls.

Group C. Twenty-four immune rats, having had previous inoculations of Flexner-Jobling rat carcinoma, given x-ray.

Group D. Twenty-four immune rats, having had previous inoculations of Flexner-Jobling rat carcinoma, kept as controls.

Group E. Forty-eight non-resistant rats kept as controls for whole experiment.

TABLE 1

FRC / 66 C	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	44	3	41	6.8	93.2	Resistant stock; no previous inoculation; given reducing exposures of x-ray
Group B	40	2	38	5.0	95.0	Resistant stock; no previous inoculation; no x-ray
Group C	19	2	17	10.5	89.5	Resistant stock; previously inoculated unsuccessfully with F. J. R. C.; given reducing exposures of x-ray
Group D	21	1	20	4.8	95.2	Resistant stock; previously inoculated unsuccessfully with F. J. R. C.; no x-ray
Group E	40	36	4	90.0	10.0	Ordinary stock. Control for experiments

Forty-eight hours after the completion of the last x-ray exposure all the groups of animals were inoculated with pieces of Flexner-Jobling rat carcinoma, 0.003 gram of tissue being inserted subcutaneously through a hollow needle; and the growths, if any, were charted at weekly intervals thereafter. The number and the percentage of takes in the animals surviving at the end of the sixth week are shown in table 1.

Group E, which was the control for the adaptability of the tumor for the whole experiment, showed 90 per cent of takes after six weeks, all of the tumors being of large size. There was practically no difference in the inoculability rate of the tumors in the immune animals, irrespective of whether they had been given x-ray treatment or not. At the end of the sixth week, as the chart shows, most of the tumors had disappeared in the radiated as well as in the non-radiated groups. The few positive takes were very slow in appearing and made poor progress in comparison with the normal growth rate of the Flexner-Jobling rat carcinoma as shown in group E. In text figure 2 the actual number of takes for the sixth week is shown graphically.

The composite lymphocyte curve of groups A and C, which were given x-ray treatment, shows (see text figure 3) an enormous reduction in the number of circulating lymphocytes compared with the control groups B, D, and E. Though the lymphocytes in the radiated groups gradually commenced to return to normal, they had not done so at the end of two weeks after the tumor had been inoculated, a time period sufficient for this tumor to have become established if it were going to grow.

The blood from the normal untreated controls for the whole experiment (group E) showed a slight initial fall in the lymphocytes, though these animals had received no x-ray or other treatment, followed by a marked rise in the number of lymphocytes. This lymphocytosis according to some of the current theories should have caused the tumors, if not to regress, at least not to continue to grow so well. Actually, however, they behaved as the Flexner-Jobling rat carcinoma does under normal conditions in any susceptible strain; and the tumors grew rapidly and well.

In this series, groups C and D, in which the percentage of takes was 10.5 and 4.8 respectively, might at first glance seem to indicate a marked increase in the number of takes after x-ray treatment. If the figures are studied, however, it will be seen

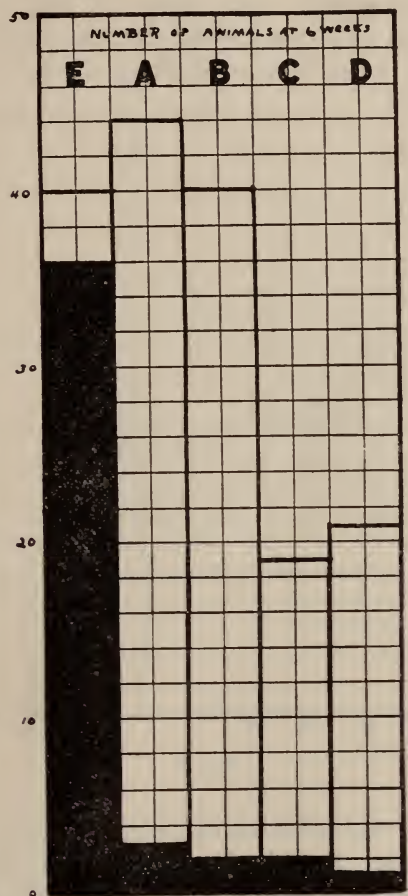

Fig. 2

that two takes to seventeen negatives in group C and one take to twenty negatives in group D do not offer a difference large enough to permit of the drawing of any deductions, such fluctuations being frequent in different series of inoculations of this tumor.

In another series the same experiment was duplicated, and as further controls, a few animals from the resistant strain, which had been previously immunized with rat embryo skin emulsion and then proved to be immune to Flexner-Jobling rat carcinoma by previous inoculations, were used. With this group it was thought advisable not to allow the lymphocytes in the radiated animals to return to normal; therefore, whenever they showed a

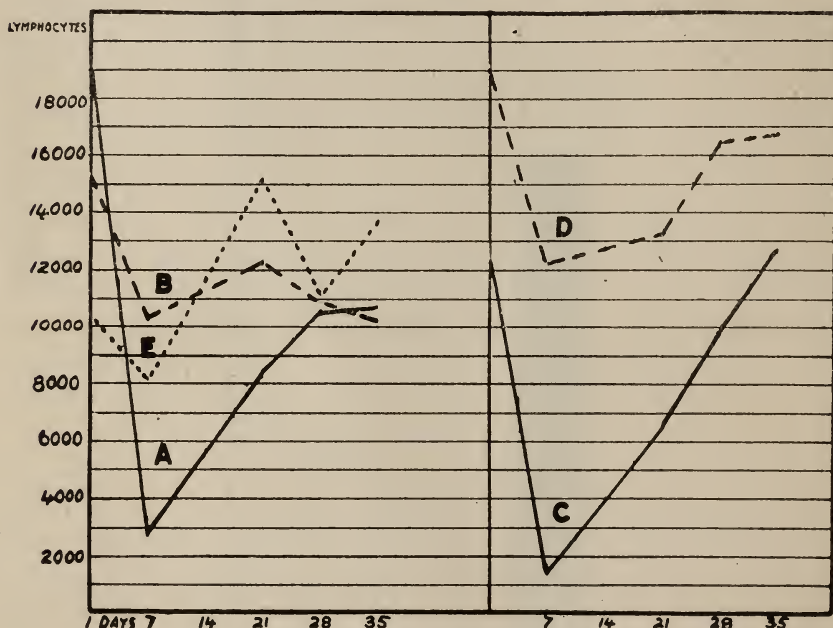

FIG. 3

tendency to do so, the reducing exposures to x-ray were repeated, so that in six weeks, they had received three separate series of reducing exposures to x-ray. The results obtained, however, were in accord with those previously described, for at the end of six weeks, in the animals which had previously been proved immune (groups C, D, F, and G) there were no takes in either the radiated or the non-radiated series (Table 2).

In groups A and B, in which A had been given x-ray for three series of irradiations and B had had no treatment, the difference

is so small as to be negligible. The lymphocyte counts of this series were taken at weekly intervals (text-figure 4) and show that by repeated radiations it is possible to keep the lymphocytes in the circulating blood very much below the normal figure.

TABLE 2

FRC / 67 G	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	15	1	14	6.7	93.3	Resistant stock; no previous inoculation; given reducing exposures of x-ray
Group B	17	1	16	5.8	94.2	Resistant stock; no previous inoculation; no x-ray
Group C	4	0	4	0	100.0	Resistant stock; previously inoculated unsuccessfully with F. J. R. C.; given reducing exposures of x-ray
Group D	10	0	10	0	100.0	Resistant stock; previously inoculated unsuccessfully with F. J. R. C.; no x-ray
Group E	13	9	3	69.3	30.7	Ordinary stock. Control for experiments
Group F	19	0	19	0	100.0	Resistant stock; previously treated with embryo skin; inoculated unsuccessfully with F. J. R. C.; given reducing exposures of x-ray
Group G	22	0	22	0	100.0	Resistant stock; previously treated with embryo skin; inoculated unsuccessfully with F. J. R. C.; no x-ray

In order to see whether by any chance a sarcoma would be influenced in any different way by the reduction of the lymphocytes in the circulating blood, the same experiment was repeated, using a rat sarcoma (R 10 of the Crocker Fund series). The animals were divided into groups as in the previous experiments; and the blood was counted before and after x-ray treatment and at frequent intervals thereafter.

The number of takes at the end of six weeks is shown in table 3. In this series as in the previous ones the composite blood curve (text-figure 5), showed a marked reduction in the number of circulating lymphocytes, but with slightly more rapid return towards the normal. In groups C and D unfortunately a majority of the rats died from some unknown cause. On that account, therefore, the experiment was repeated on a somewhat larger scale, with the results shown in table 4. Here, however, although several months and a number of generations of the tumor had elapsed between the two series of experiments, the results were the same. When the resistant animals were rendered

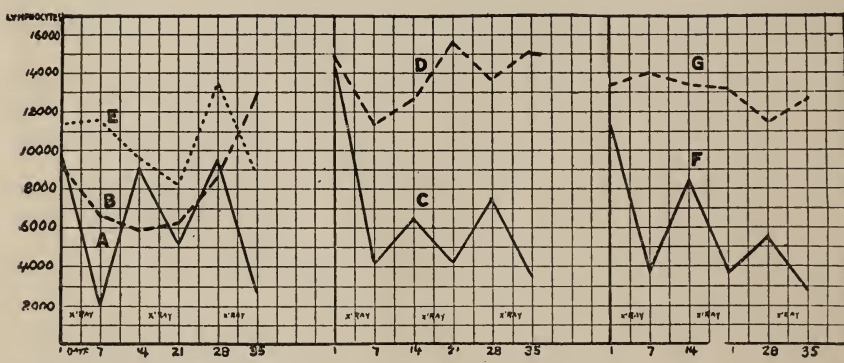

Fig. 4

immune by previous doses of tumor and were then given reducing doses of x-ray, there was no difference between the treated animals and the controls as regards the number of growths at the end of five weeks. In both groups the results were 100 per cent negative. In all the series, however, except in the case of the Jensen rat sarcoma, when the resistant stock had not been rendered immune by previous inoculations the animals treated with x-ray showed a slightly higher percentage of takes than did the animals which had not been given x-ray. It would seem, however, that the importance of this experiment lies in the results obtained from the *immune* animals, for it is only from these that we can draw accurate conclusions as to the rôle played by the lymphocyte in tumor production. In going over the chart

TABLE 3

R 10 / 23 E	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	21	3	18	14.2	85.7	Resistant stock; no previous inoculation; given reducing exposures of x-ray
Group B	24	3	21	12.5	87.5	Resistant stock; no previous inoculation; no x-ray
Group C	8	0	8	0	100.0	Resistant stock; previously inoculated unsuccessfully with R 10; given reducing exposures of x-ray
Group D	3	0	3	0	100.0	Resistant stock; previously inoculated unsuccessfully with R 10; no x-ray
Group E	22	21	1	95.4	4.5	Ordinary stock. Control for experiment

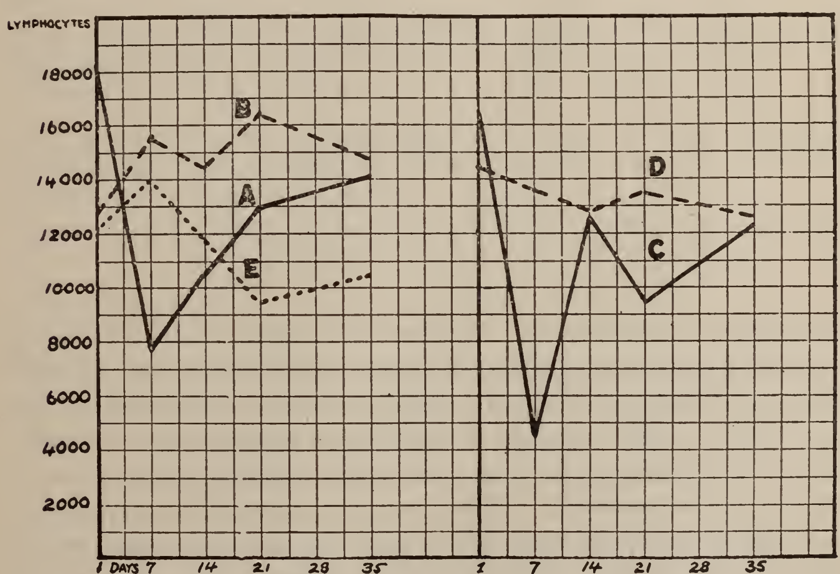

FIG. 5

books in the Crocker Laboratory for the last six years, there was found in all our series of the Jensen rat sarcoma a variation in the number of takes. The difference in the takes in groups A and B, therefore, though it may look suggestive, certainly does not warrant the drawing of any deductions, for greater fluctuations occurred in routine transplantations.

In this sarcoma series, as in the carcinoma, the results do not show the effects of x-radiation reported by Murphy and Morton or

TABLE 4

$\frac{\text{R 10}}{\text{30 B}}$	NUMBER OF RATS	POSITIVE	NEGATIVE	PERCENTAGE OF TAKES	PERCENTAGE NEGATIVE	EXPLANATION
Group A	66	42	24	63.6	36.4	Resistant stock; no previous inoculation; given reducing exposures of x-ray
Group B	68	37	31	54.5	45.5	Resistant stock; no previous inoculation; no x-ray
Group C	53	0	51	0	100.0	Resistant stock; previously inoculated twice unsuccessfully with R 10; given reducing exposures of x-ray
Group D	65	0	65	0	100.0	Resistant stock; previously inoculated twice unsuccessfully with R 10; no x-ray
Group E	60	59	1	98.3	1.7	Ordinary stock. Control for experiment

by Mottram and Russ (4). The latter investigators found, during a series of experiments with the Jensen rat sarcoma, that animals which had been previously proved immune to inoculations of that tumor would, when given sufficiently large doses of x-ray to reduce the lymphocyte count markedly, permit the growth of the Jensen rat sarcoma. They, however, gave their x-ray doses in one prolonged treatment rather than repeated over a protracted period. They deduce from their experiments that some of the original grafts persisted for a longer time in the irradiated animals than in the controls, and that when the tumor did grow it

was delayed in making its appearance and almost invariably receded; but in our series this also occurred when x-ray had not been given, and the difference in the tumors which survived and grew in groups A and B show nothing to indicate any marked variation in favor of those whose lymphocytes had been reduced by x-ray radiation. The number of animals used by them is too small to permit the drawing of general conclusions as to the relationship between the x-rays and the presence or absence of immunity, except with the Jensen rat sarcoma and in the particular growth phase at the time the work was done. For example, the record charts of the Crocker Fund show that an occasional rat immune to inoculation with this tumor may give a good growth on reinoculation. In this laboratory, also, we do not find that the Jensen rat sarcoma when inoculated into rats grows almost invariably, as it does in England; and a careful analysis of 5543 animals extending over a period of six years shows the average number of takes to have been only 71 per cent. The number of takes varied greatly according to the months, from 45 per cent in July to 77 per cent in August.

In order, therefore, to test the results of Mottram and Russ, and to add another type of sarcoma to our experimental series, a group of rats was given x-ray treatment under conditions similar to those of the foregoing experiments, and then inoculated with the Jensen rat sarcoma. The results are shown in table 5. In group A, comprising animals of resistant stock whose lymphocytes had been reduced by exposures to x-ray, there were fewer takes than in group B, made up of animals of resistant stock which had not been exposed to x-ray; whereas in the immune animals the results were 100 per cent negative, whether they had been exposed to reducing doses of x-ray or not. The blood pictures were the same as those shown in the above charts, and have, therefore, been omitted. In all these experiments, it was found, just as was reported by Mottram and Russ, that occasionally a small tumor in the radiated animals would persist longer than one in the controls; but even when the lymphocytes were kept low and not allowed to return to normal, the tumor ultimately disappeared. It may be that the

introduction of a graft of tumor into an animal acts as an antigen, producing a small amount of a cytotoxic antibody, and thus causes regression of small, poorly established tumors; and it is also possible that the action of the x-ray on such animals is to hold the production of the antibody in check, as has been shown by Hektoen. This inhibition of antibody formation may permit a pin-point or small growth to remain for a time; but from our results and those of others it does not seem that this persistence

TABLE 5

JRS / 95 A	Number of rats	Positive	Negative	Percentage of takes	Percentage negative	Explanation
Group A	36	15	21	41.7	58.3	Resistant stock; no previous inoculation; given reducing exposures of x-ray
Group B	61	30	31	49.1	50.9	Resistant stock; no previous inoculation; no x-ray
Group C	19	0	19	0	100.0	Resistant stock; previously inoculated twice unsuccessfully with J. R. S.; given reducing exposures of x-ray
Group D	43	0	43	0	100.0	Resistant stock; previously inoculated twice unsuccessfully with J. R. S.; no x-ray
Group E	59	41	18	69.8	30.2	Ordinary stock. Control for experiment

is due to the lowering of the lymphocytes in the blood, as ultimately these small growth disappear despite the lowering of the cell count.

SUMMARY

In animals which are naturally resistant or artificially immune to certain strains of rat carcinoma and sarcoma, a marked reduction in the circulating lymphocytes produced by x-ray treatment is not followed by an appreciable decrease in their immmnity to tumor inoculation.

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FLUCTUATIONS IN INDUCED IMMUNITY TO TRANSPLANTED TUMORS

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Investigators in cancer research who have performed any large number of experiments in which an attempt has been made to induce immunity against transplantable animal tumors have probably been impressed by the variations in the percentages of actual immunity produced by the methods commonly employed. Perhaps it has been their experience to obtain wide differences in their results at different periods in the life of a particular tumor even after using the same technic and immunizing material. If they have had occasion to review the published experiments in the same field they will have observed that these variations have also occurred in the work of other men.

As illustrative of these variations, we present Chart 1, which depicts graphically the results obtained in rats immunized with 0.05 gram of rat fetal skin, 0.05 gram of rat spleen emulsion and 1 cc. of rat blood cells respectively, and inoculated ten days later with the Flexner-Jobling rat carcinoma in dosage of 0.003 gram.[1] The relatively small dose of immunizing material was intentionally used to secure, if possible, a more delicate reaction. It will be noted that an immunity varying from 3 to 70 per cent was obtained, and that these variations occurred irrespective of the kind of immunizing material used. In these groups there

[1] In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

was no relationship between the percentage of natural immunity as present in the control groups and that of induced immunity in the immunized groups.

It is, of course, obvious that if variations in induced immunity are at all common their occurrence introduces a grave factor of error in many of the experiments in which immunity plays a part. It is possible, for example, for one investigator by using

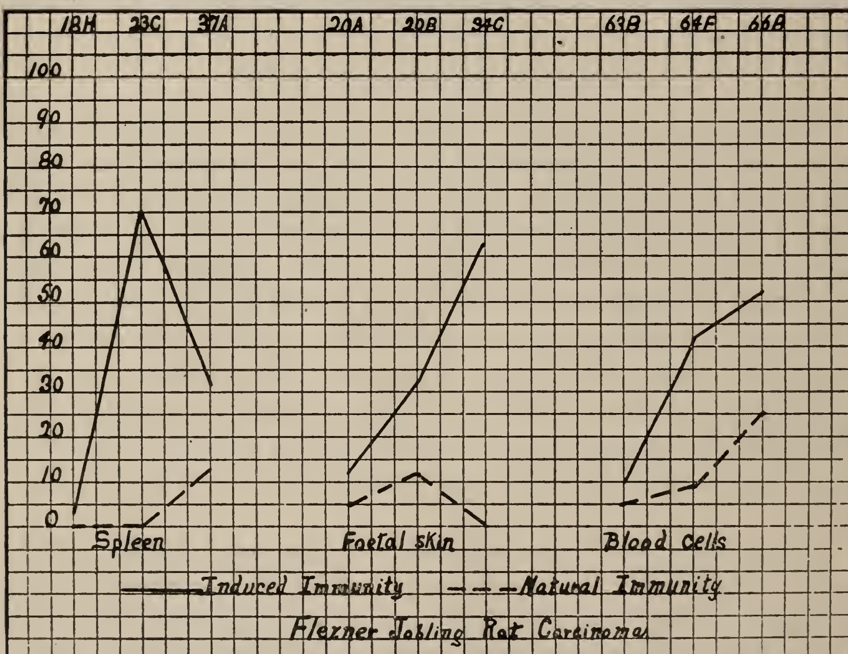

CHART 1. FLUCTUATIONS IN PERCENTAGES OF INDUCED AND NATURAL IMMUNITY WITH FLEXNER JOBLING RAT CARCINOMA

one kind of immunizing material to obtain no immunity against a particular tumor, while another by employing the same kind of immunizing material and tumor strain may obtain a high percentage of immunity. The publication of the two experiments with directly opposite results would tend to create confusion unless the occurrence of such extraordinary fluctuations were fully appreciated.

The object of the experiments recorded in this paper was to establish the fact that fluctuations in induced immunity are a frequent occurrence, and to determine, if possible, the factors responsible for them so that in the future they might be eliminated. In our investigations, three tumor strains were used; the Flexner-Jobling rat carcinoma, the Buffalo rat sarcoma, and the English mouse carcinoma 63.[2]

In order to exclude as far as possible the factor of variation in technic, a standard was decided upon and rigidly followed. Immunity was induced by subcutaneous injection of homologous splenic pulp emulsion in dosage of 0.07 gram. Ten days after immunization, the animals were inoculated subcutaneously with 0.003 gram of the tumor strain. Immunization and inoculation were performed by the same person in all experiments in order to eliminate the factor of individual variation in technic. The tumors used for inoculation were derived whenever possible from the most recent generation and were eighteen days old. The growth of the tumor was charted for the first time ten days after inoculation and weekly thereafter. The percentages are based on conditions as present on the twenty-fourth day of tumor growth.

Twenty-four animals were used in each series, the controls being a similar number of normal non-immunized animals derived from the same stock. In the entire experiment, animals of about the same age, development, and state of health were chosen.

Chart 2 presents in graphic form the percentages of immunity obtained with the Buffalo rat sarcoma by following the outlined technic. The percentages varied in different generations from 18 as a low, to 95 as a high point. Although the percentages of natural immunity also varied the variations bore no relation to those of induced immunity.

While sarcoma is notoriously difficult to immunize against, the criticism may perhaps be advanced that the fluctuations noted are dependent upon the health of the animals, it being

[2] This laboratory is indebted to Dr. Murray, Director of the Imperial Cancer Research Fund of London, England, for the last named tumor strain.

well known that animals in poor health are less susceptible to tumor inoculation than are healthy animals. One index of the health of any given group of organisms is the mortality rate. This rate has been plotted as a curve in this and in the succeeding series to be later described. It is true that with the Buffalo

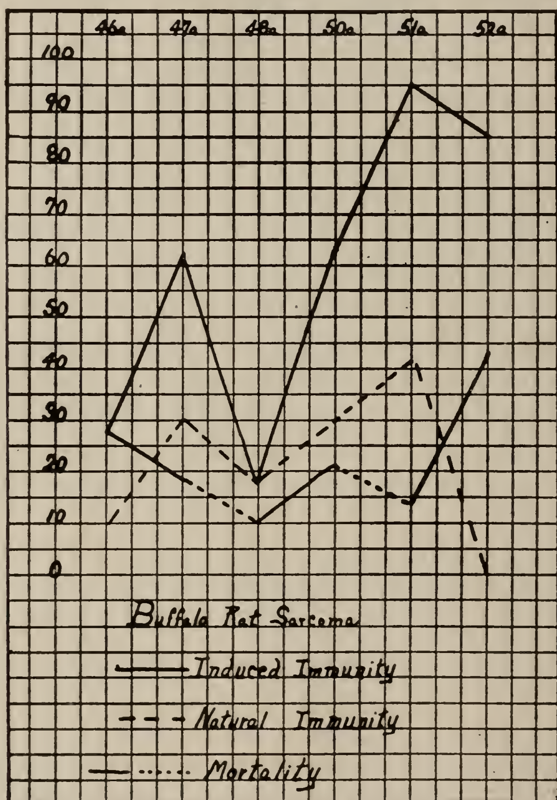

CHART 2. FLUCTUATIONS IN THE PERCENTAGES OF INDUCED AND NATURAL IMMUNITY OF THE BUFFALO RAT SARCOMA

tumor, with a mortality of 10 per cent, the immunity induced was 18 per cent and that when the mortality was 42 per cent the immunity reached 95 per cent. For this series, and for this series only, it is apparently true that when the animals were healthy, as evidenced by a low mortality, the percentage of

induced immunity was low, while at another time when the health of the animals was poor, with a high mortality, the immunity was also high.

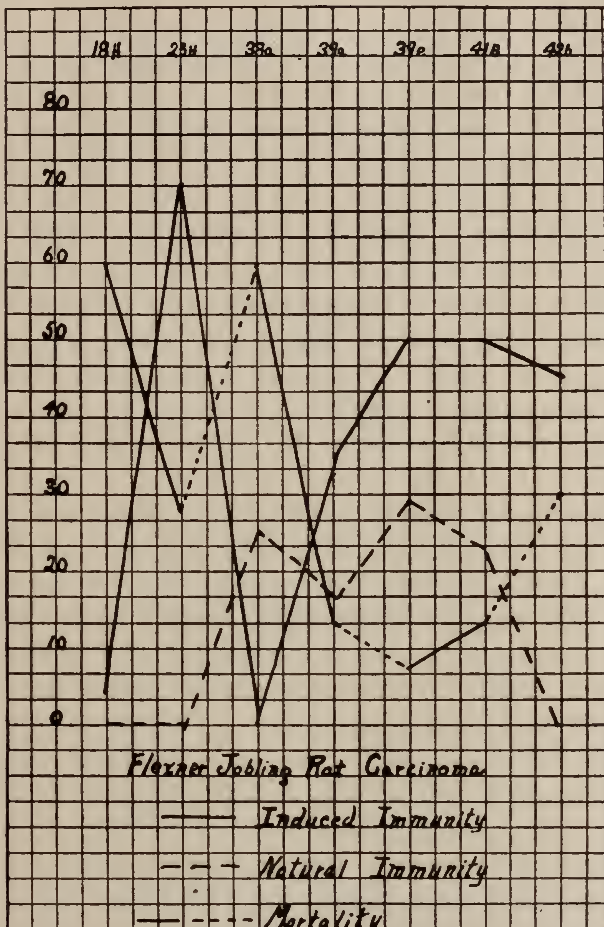

CHART 3. FLUCTUATIONS IN THE PERCENTAGES OF INDUCED AND NATURAL IMMUNITY OF THE FLEXNER JOBLING RAT CARCINOMA

When the Flexner-Jobling tumor was used, variations again occurred as is shown in chart 3. In this series the low point was zero while the high point was 70 per cent. The mortality

percentages in this series, however, present the directly opposite relationship apparent with the Buffalo tumor, for on two occasions when the death rate was 60 per cent, in other words, when the health of the animals was poor, there was practically no immunity obtained.

Pitzman (1), in explanation of these fluctuations, has advanced the hypothesis that immunity is due to bacterial contamination of either the tumor or the immunizing material, or possibly of both. This suggestion has been, however, shown to be without foundation by Tyzzer (2) and Woglom (3). But to rule out any possible source of error two generations in which the mouse carcinoma 63 was employed were immunized with mouse fetal skin emulsion instead of splenic tissue, and cultures were made of the immunizing material and of the tumor tissue. All the cultures proved sterile. Bacterial contamination can, therefore, be eliminated. Nevertheless, as is shown in chart 4, there was a marked variation in the percentage of immunity obtained.

One other factor that remains to be considered is the purity of breed of the animals used. The influence of this factor upon the growth of first and even subsequent transplants of tumor is well known; for example, tumors from Danish mice may not grow in mice bred in Germany. It was thought possible that a similar circumstance might explain the variations encountered in inducing immunity. To control this element the mouse tumor 63, which, as we have stated before, is of English derivation, was inoculated into pure bred English mice, immunity being induced in the usual manner by means of mouse spleen emulsion. As will be noted in chart 5, the variations encountered were slight, the high point being 100, the low point being 88 per cent. Variations of but twelve points in a biological experiment of this kind can be disregarded, as they fall within the limits of error.

It is evident from the data presented that variations of marked degree do occur in the percentages of induced immunity obtained after the commonly accepted methods of procedure. The importance of the recognition of these fluctuations is self-

evident, since their occurrence markedly impairs the value of any deductions from experiments in which they have not been considered and eliminated.

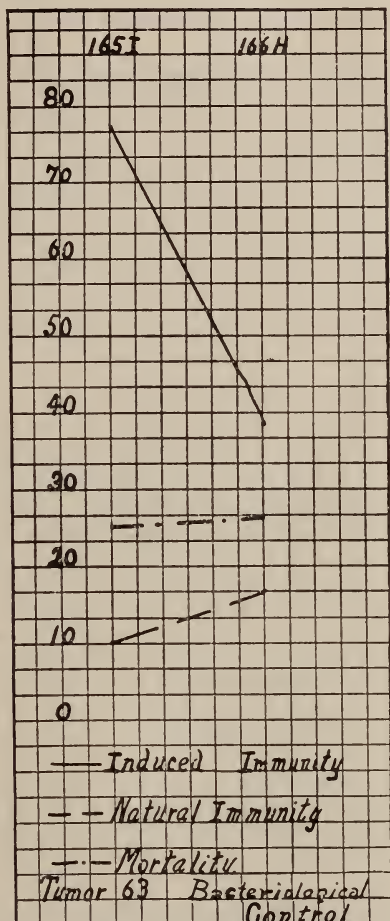

Chart 4. Variations in Induced Immunity with Tumor 63, When Both Tumor and Immunizing Material Are Shown to Have Been Sterile

The factor responsible for these variations is at first glance not quite evident. Variations in technic, particularly in minor alterations in the dosage of the immunizing material, have been

eliminated. Bacterial contamination, with a possible destruction of the inoculated fragments of tumor, is also not sufficient to explain the variations noted, since fluctuations occurred when both immunizing tissue and tumor were sterile. The type of tissue used for immunization cannot be held responsible, for

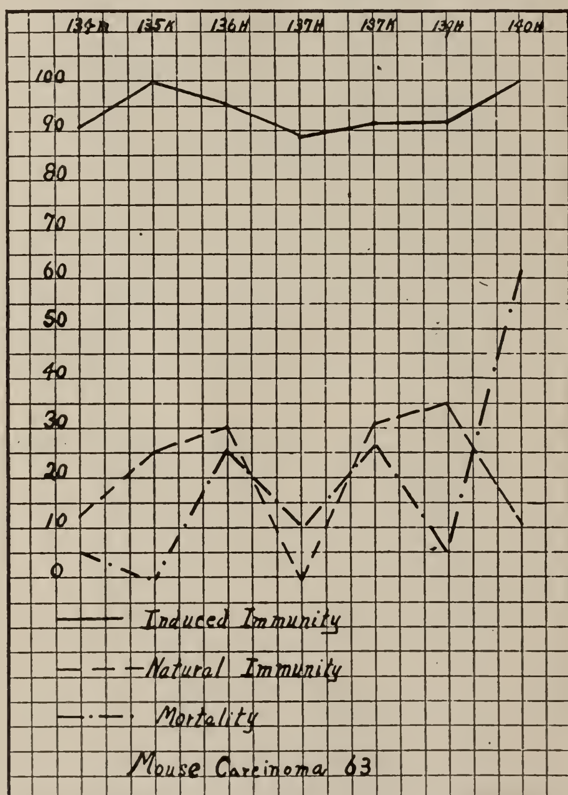

Chart 5. Fluctuations in the Percentages of Induced and Natural Immunity of Tumor 63

the variations were noted with blood cells, spleen emulsion, and fetal skin emulsion. Epithelial and connective tissue tumors of both the rat and the mouse showed the variations. However, when the purity of breed of the experimental animals was assured then the variations were of such minor type that they

could be disregarded. Russell (4) obtained fairly constant results in his published experiment in induced immunity by using a pure strain of mice. The most plausible explanation, therefore, is that the biological relationship between tumor, immunizing material, and host is a very delicate one and that unrecognizable differences between different strains of animals are sufficient to create the wide variations noted.

In any experiment in which immunity plays a part these variations should be eliminated or rendered as small as possible by first transplanting the tumor for a number of successive generations into a pure strain of animals and then using animals of the same strain for the immunity experiments. It is only by doing this that accurate deductions from experimental results will become possible.

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FLUCTUATIONS IN CONCOMITANT IMMUNITY

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The variations in the percentage of induced immunity which we obtained by the methods commonly used in immunizing animals against transplanted tumors have been discussed in another publication (1). In the present paper it will be shown that fluctuations of a similar character occur in concomitant immunity, following a previous inoculation with either a receding or a progressively growing tumor.

In investigations which have been recorded elsewhere (2) the action of degenerative tumor products upon immunity was studied, it being our original intention in planning these experiments to see if tumors which do not produce concomitant immunity could be made to do so by an increase in the products of tumor degeneration. In this connection it will be remembered that Russell (3) has divided tumors into two classes, those which immunize against subsequent grafts of a tumor of the same strain (concomitant immunity) and those which do not. He presented the mouse carcinomata Twort and 63[1] as typical of those which do not produce concomitant immunity. The results of our experiments referred to above are of interest in this connection because of the fact that in our hands these two tumor strains did produce concomitant immunity. Chart 1 copied from Russell's article shows his results with the Twort tumor, while Chart 2 depicts our results with the same strain. Chart 3 is a reproduction of Russell's results with tumor 63, while charts 4 *a*, 4 *b* and 4 *c* represent our results with the same strain. It will

[1] This laboratory is indebted to Dr. Murray, Director of the Imperial Cancer Research Fund of London, England, for the tumor strains Twort, 63, and 206.

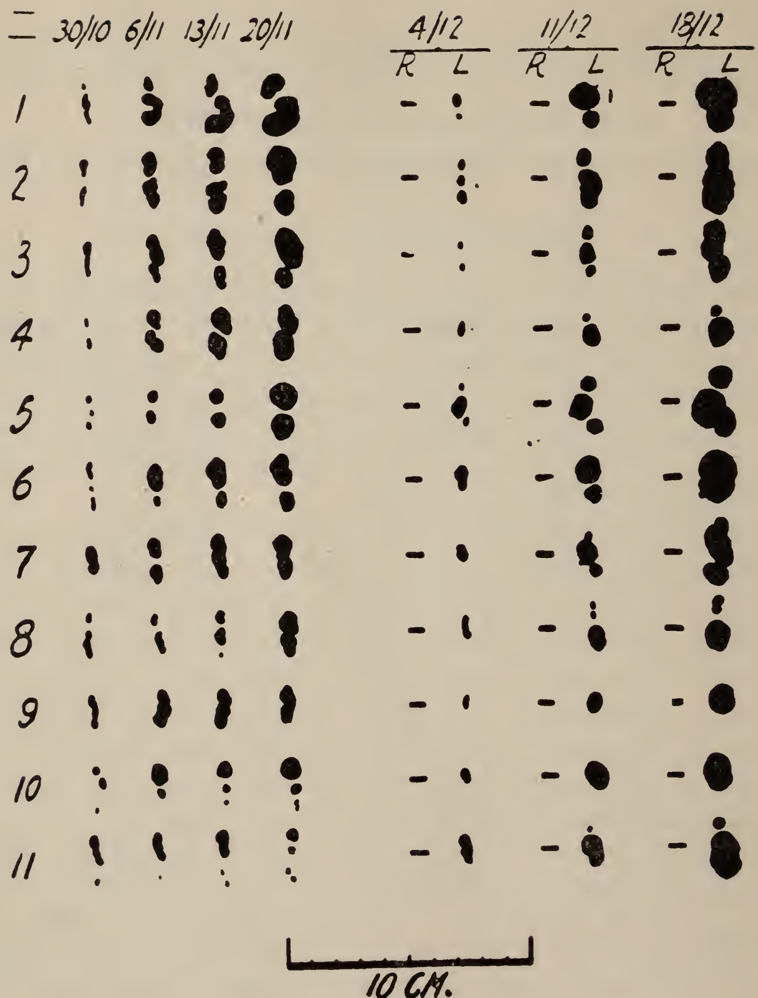

Chart 1. Twort Tumor in Non-Immunizing Phase (After Russell)

Mice inoculated in right axilla 20/10. Tumors excised 22/11 and the animal reinoculated in left axilla 24/11.

be noted that our results do not agree with those obtained by Russell.

Prime, of this laboratory, unaware of our experiments and while working on another problem, also obtained results at variance with those of Russell while using the British mouse carcinoma 206 as an immunizing agent. According to Russell (4)

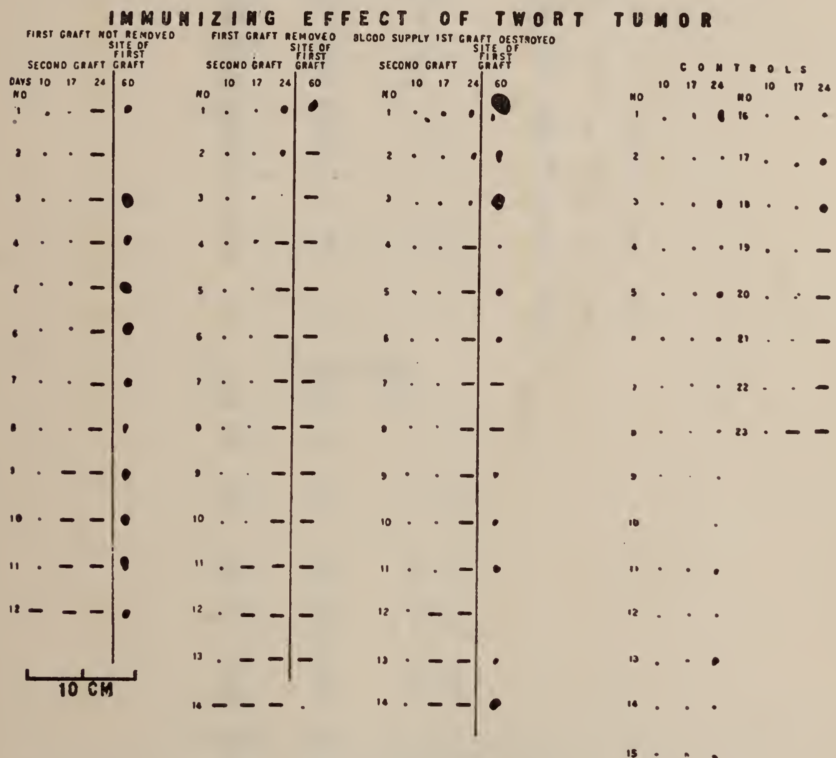

CHART 2. AN EXPERIMENT WITH TWORT TUMOR SHOWING A HIGH PERCENTAGE OF CONCOMITANT IMMUNITY

this tumor strain immunizes against a subsequent inoculation of the British mouse carcinoma 63 and the records of his experiment show an immunity of 100 per cent. Prime, on the contrary, in an experiment heretofore unrecorded (chart 5), obtained but 23 per cent immunity in one generation and 30 per cent in the next, a variation of approximately 70 from Russell's figures.

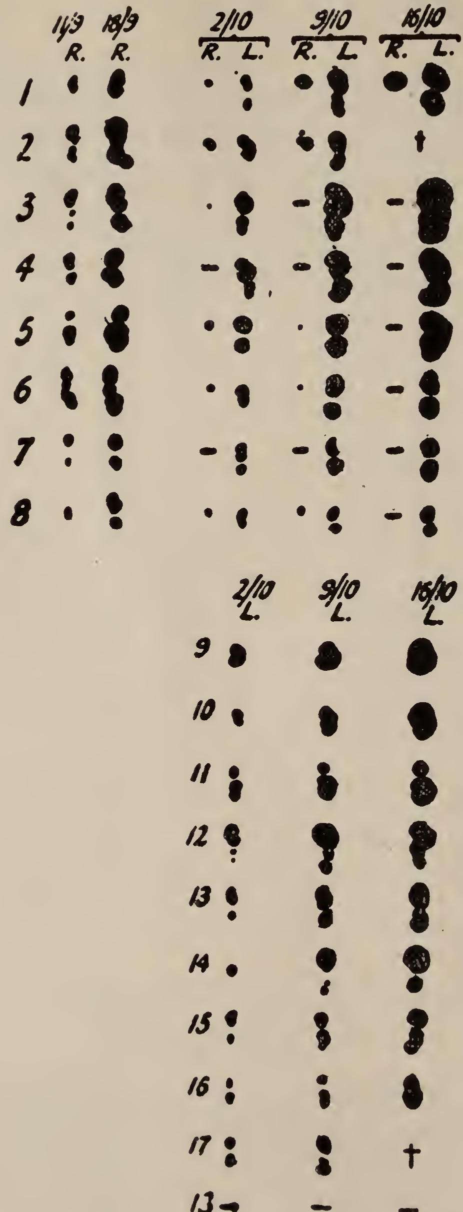

Chart 3. Tumor 63 in Non-Immunizing Phase (After Russell)

Mice 1 to 8 inoculated in right axilla 31/8. Tumors excised 19/9 and the mice reinoculated in left axilla 21/9. Mice 9 to 18 controls for second inoculation.

The objection may be raised that these are isolated occurrences readily explained by the fact that English tumors have been transplanted into animals of American stock. In answer to this objection, we present similar results which were obtained with the Buffalo rat sarcoma. Chart 6 shows this tumor in a

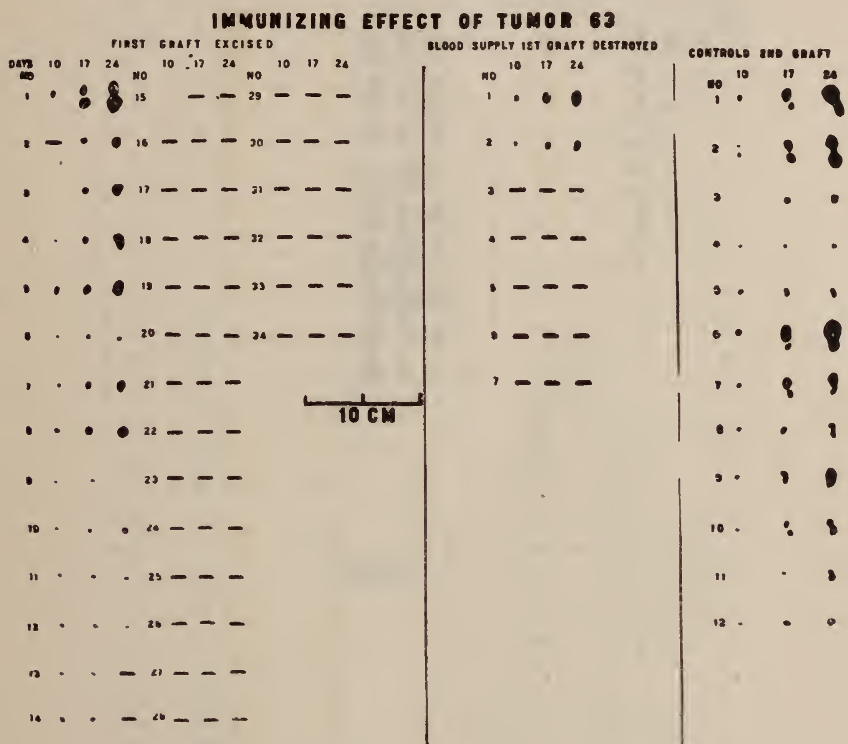

CHART 4 A. AN EXPERIMENT IN WHICH TUMOR 63 GAVE RISE TO A HIGH DEGREE OF CONCOMITANT IMMUNITY

phase when it did induce concomitant immunity, while chart 7 shows it at a time when that power was lost.

The above examples have been cited to show that fluctuations in concomitant immunity do occur. The experiments recorded in this paper were planned to determine the frequency of this occurrence and to discover, if possible, the factor or factors which are responsible for these variations. As in our experi-

ments in induced immunity, three tumor strains were used, the Buffalo rat sarcoma, the Flexner Jobling rat carcinoma, and the English mouse carcinoma 63.

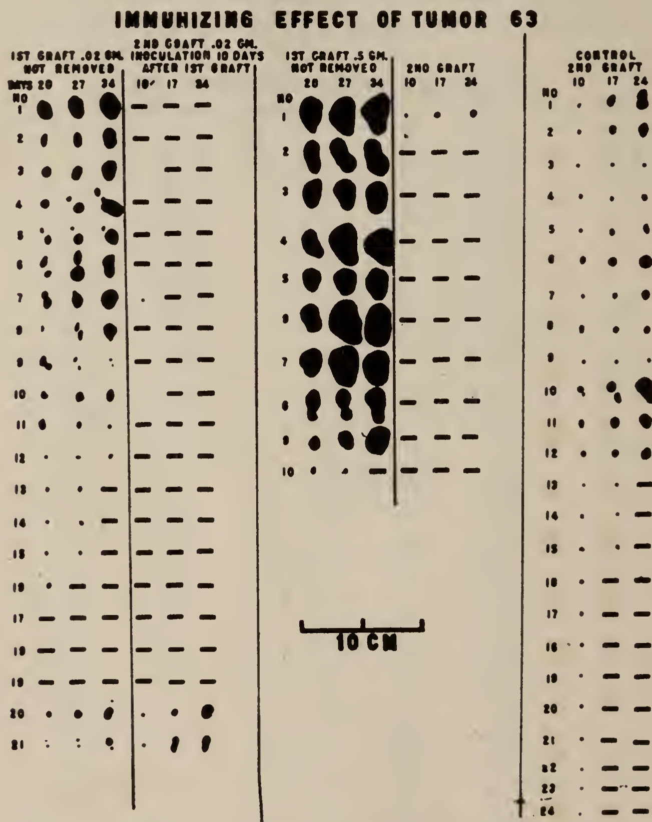

CHART 4 B. ANOTHER EXPERIMENT IN WHICH TUMOR 63 GAVE RISE TO A HIGH DEGREE OF CONCOMITANT IMMUNITY

To eliminate variations due to differences in technic, a standard was decided upon and all inoculations were performed by the same operator. Animals were inoculated with 0.003 gram[2] of

[2] In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram, respectively.

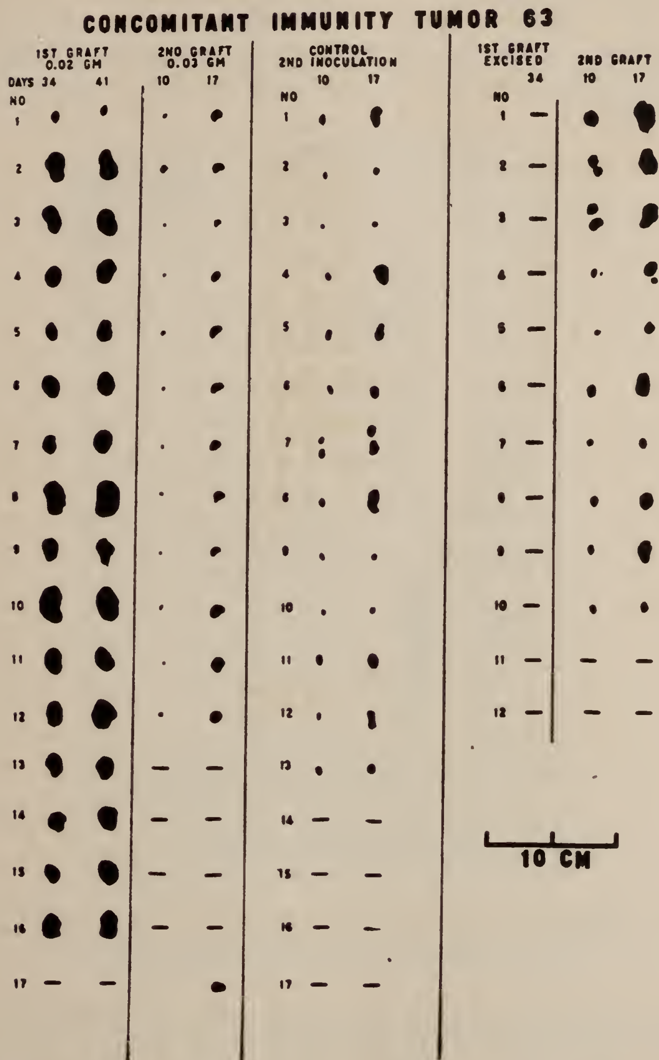

CHART 4 C. AN EXPERIMENT IN WHICH TUMOR 63 CLOSELY FOLLOWED RUSSELL'S EXPERIENCE

the given tumor strain, the tumors for inoculation being derived, whenever possible, from the preceding generation, and being 18 days old. Eighteen days after inoculation, the animals were reinoculated in the tissues of the opposite side of the body with a similar dose of tumor of the same strain. The tumor used

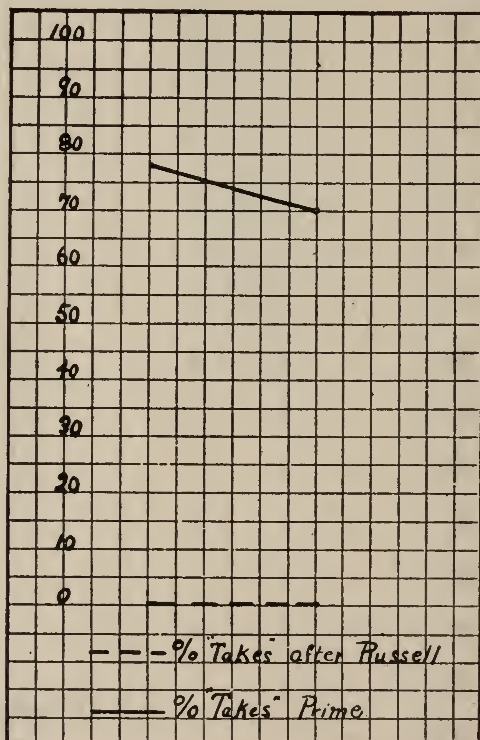

Chart 5. A Comparison of the Percentages of Takes Obtained by Russell and by Prime Using the Tumor 206 and 63

for the second inoculation was selected from the tumors resulting from the first inoculation. Twenty-four animals were used for each generation, and each set of inoculations was controlled by a normal group of 24 animals. In the entire experiment animals of about the same age, development, and state of health were chosen. The tumors were charted for the first time ten

days after inoculation and weekly thereafter, and our figures are based on the condition present on the twenty-fourth day of growth of the second graft.

Concomitant immunity, as observed through six generations of the Buffalo rat sarcoma (chart 8), ranges between 100 per

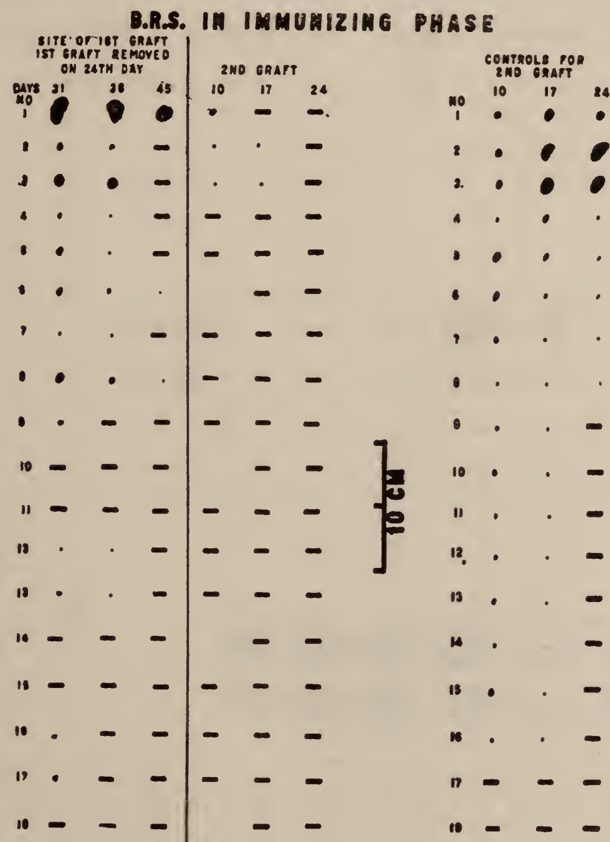

CHART 6. BUFFALO RAT SARCOMA IN A PHASE WHERE IT INDUCED CONCOMITANT IMMUNITY

cent and 65 per cent, a variation of 35 per cent. No relationship is apparent between the curves of concomitant and those of induced or natural immunity in the same generations (chart 9).

Although the power to produce concomitant immunity is not

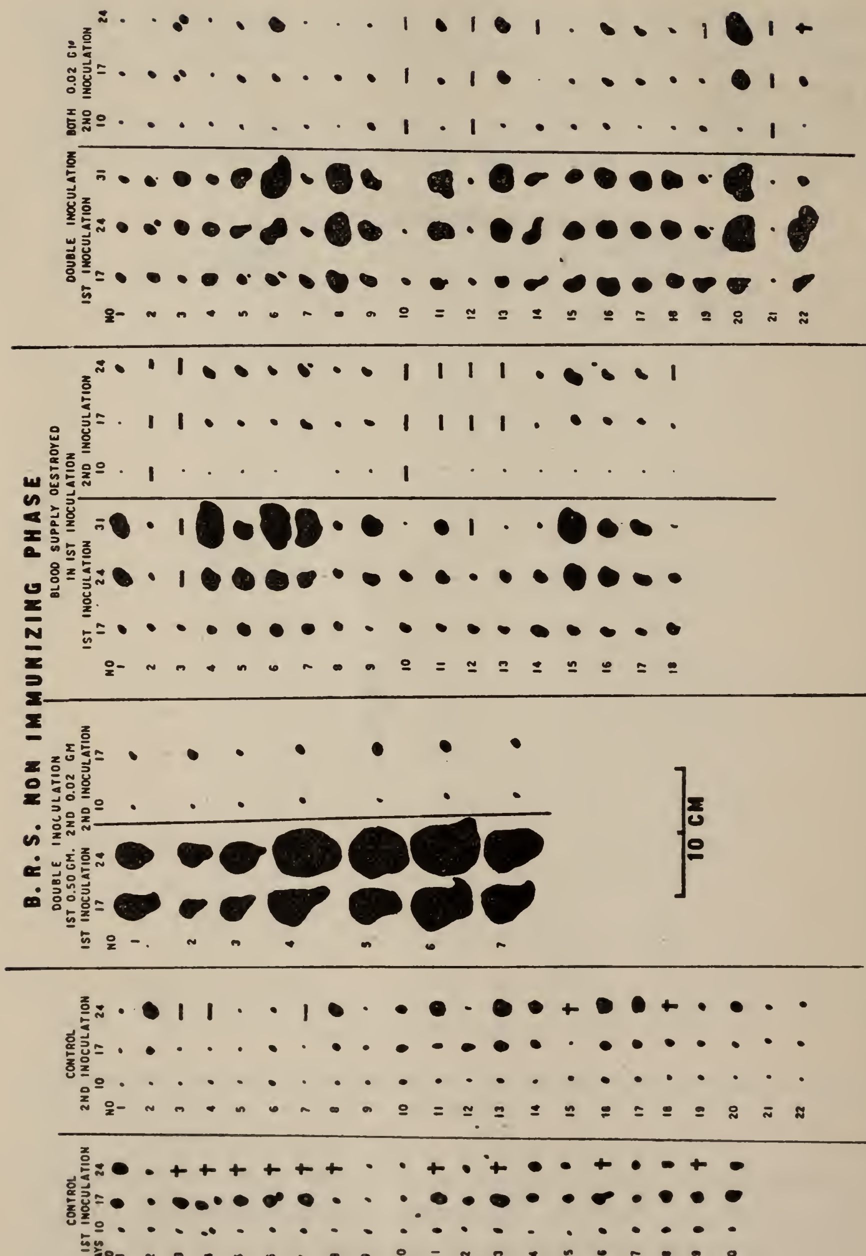

Chart 7. Buffalo Rat Sarcoma in a Phase Where it Did Not Induce Concomitant Immunity

CHART 8. A COMPARISON OF THE PERCENTAGES OF CONCOMITANT IMMUNITY AND MORTALITY WITH THE BUFFALO RAT SARCOMA

so great, the variations observed through seven generations of the Flexner tumor (chart 10) are similar to those noted in the Buffalo tumor, the high point of immunity being 70 per cent,

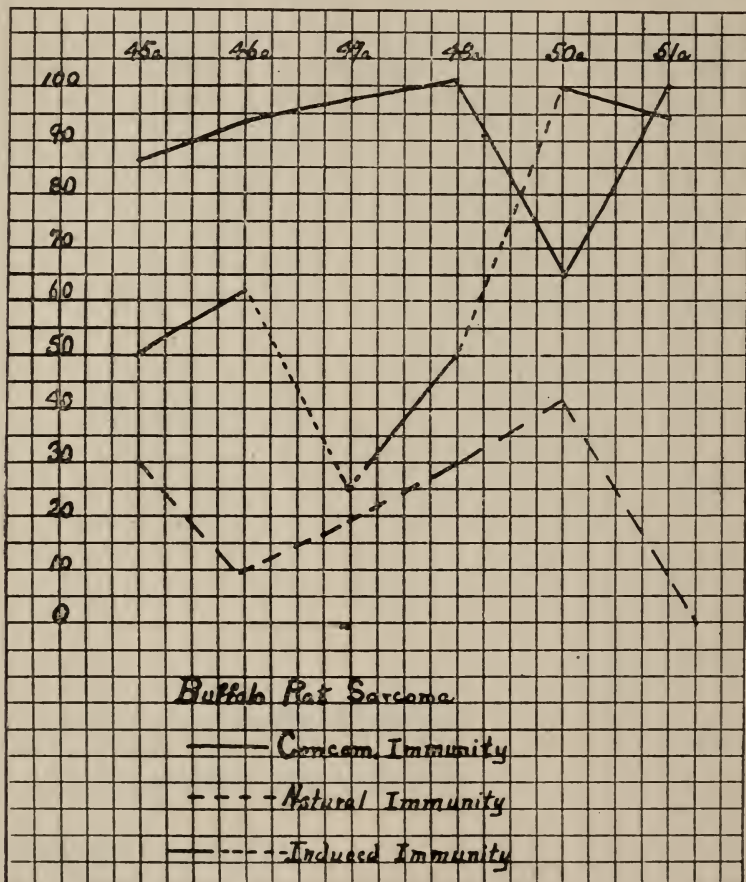

Chart 9. A Comparison of the Variations in Percentages of Concomitant, Induced, and Natural Immunity in the Same Generation of the Buffalo Rat Sarcoma

while the low point was 32. With this tumor, also, the concomitant immunity is apparently independent of the induced and the natural immunity (chart 11).

To obviate the factor of familial or racial tendencies, a factor which is known to influence not only the percentage of takes in transplanted tumors, but also, as we have shown, the percentage of induced immunity, a pure strain of mice was used for the

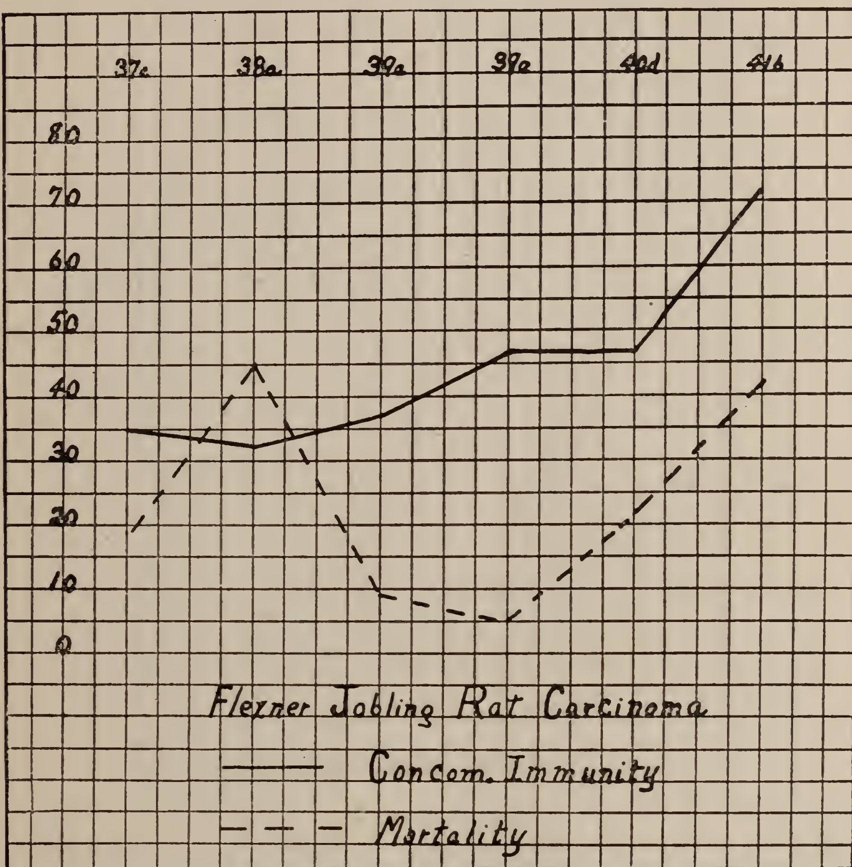

CHART 10. A COMPARISON OF THE PERCENTAGES OF CONCOMITANT IMMUNITY AND MORTALITY WITH THE FLEXNER JOBLING RAT CARCINOMA

mouse carcinoma 63, both tumor strain and animals being of English stock. Through eight generations of this tumor (chart 12) even more marked and more frequent fluctuations occurred than with the previous two strains, the low being 21 and the

high being 60 per cent. As with the other strains, no relationship can be established between the variation in induced, concomitant, and natural immunity (chart 13).

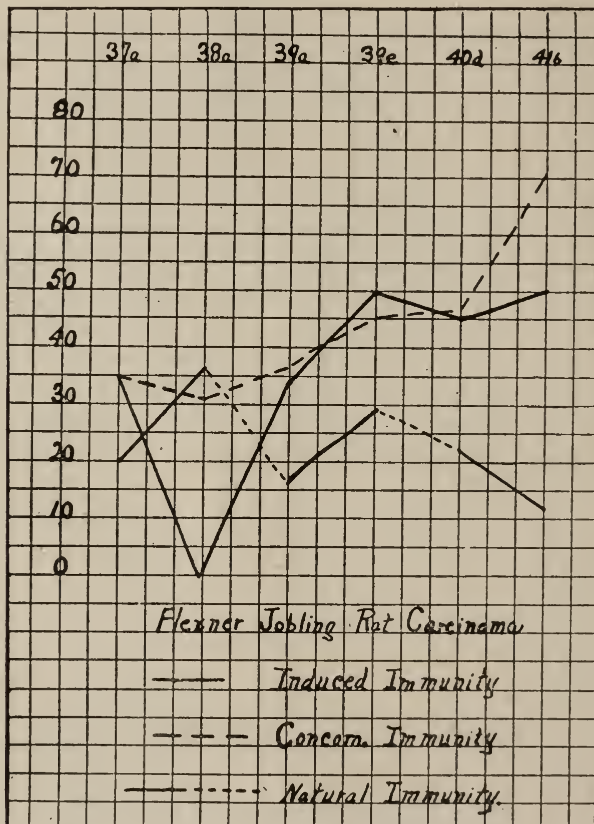

Chart 11. A Comparison of the Variations in Percentages of Concomitant, Induced, and Natural Immunity in the Same Generation of the Flexner Jobling Rat Carcinoma

In charts 8, 10, and 12, the mortality curves and the concomitant immunity curves for each of the three tumor strains are presented. It is evident that there is no relation between the health of the animals, as indicated by the mortality curve, and the percentage of concomitant immunity.

From the experiments which have been described in the previous paragraphs, it is evident that fluctuations in concomitant immunity frequently occur with both epithelial and connective tissue neoplasms in mice and rats. It will be recalled that analagous variations in induced immunity were found to be due to differences in the host strain. This, however, is not true of con-

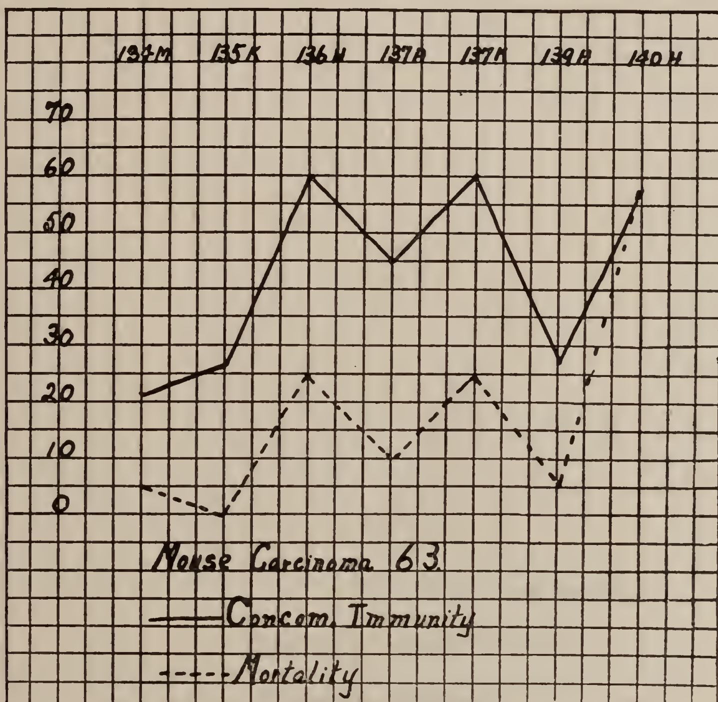

Chart 12. A Comparison of the Percentages of Concomitant Immunity and Mortality with Tumor 63

comitant immunity. Neither can the variations be connected with similar fluctuations in either induced or natural immunity, apparently somewhat different forces being operative in each instance. Variations in technic have been eliminated by following the standard laid down in an earlier paragraph. By exclusion, therefore, the vacillations must be due to differences in the tumor itself. If, as Bashford has suggested in explaining

another phase of tumor biology, these variations are due to fluctuations in the growth energy of the tumor, then growth energy must be measured not by the infectivity of a tumor, as indicated by the number of takes, but by the rapidity of growth of a

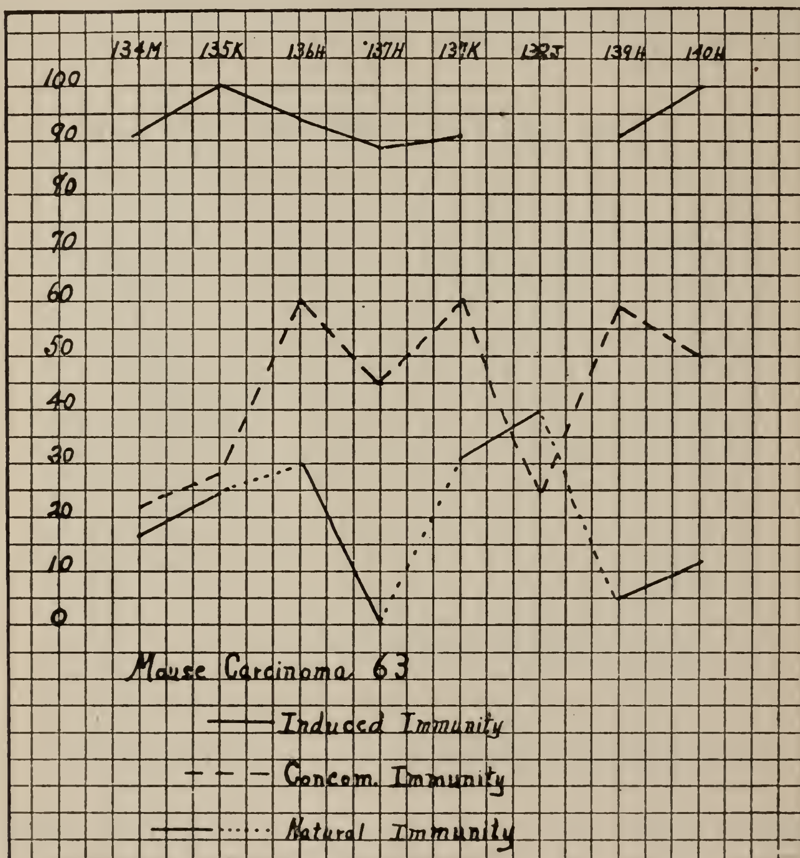

Chart 13. A Comparison of the Variations in Percentages of Concomitant, Induced, and Natural Immunity in the Same Generation of Tumor 63

single given tumor. Though it is probably impossible to establish a standard for size or weight of a given tumor strain after a fixed period of growth, it appears from our experiments that concomitant immunity occurred most often when the individual

tumor grew slowly but steadily, and least often when the tumor grew rapidly. Whatever the cause of the variations noted, their presence is proof of the inconstancy of tumors as immunizing agents.

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A BASAL-CELL EPITHELIOMA OF THE RAT

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From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

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The tumor to be described arose spontaneously in a young male rat, about five months old, weighing 60 grams. When first observed, it appeared as a flattened oval mass, about 2.5 by 1.5 cm. in diameter, occupying the skin of the left thoracic region, and resembled an epithelioma such as occurs frequently on the human face. At the center the tumor was ulcerated, with an uneven granular surface. The margins of skin about this were nodular, raised, thickened, and slightly undermined, and of a reddened shiny appearance which shaded rather abruptly into the normal tissues. The consistency of the mass was distinctly firm, and it was freely movable on the deeper parts.

The tumor was removed by operation, and the major part of it was inoculated into 204 rats and, in two places, into its bearer. Serial paraffin sections which were made of the remaining tissue showed the following histological characteristics:

The tumor was in direct connection with the skin (fig. 1) and extended into the subcutaneous tissue but not into the muscle; it was composed of rounded and irregular alveolar masses of cells having the same staining and morphological characteristics as the basal cells of the epidermis and skin appendages (fig. 2). There were all gradations from typical basal cells to those of a spindle shape. The cells were of small size, with a deeply staining nucleus and relatively small amounts of protoplasm giving the alveoli a very dark appearance. In places these cells were in strands and whorls resembling a spindle-cell sarcoma (fig. 3). Here and there, in the center of cellular areas,

were glandular (fig. 4) or cystic structures filled with cell débris and lined by squamous cells in layers; there was, however, no production of keratin. The overlying skin was not abnormal except at one point where the strands of tumor cells and the basal layer of the epidermis were continuous. Staining with the Bielschowski method showed no intercellular collagen fibrils.

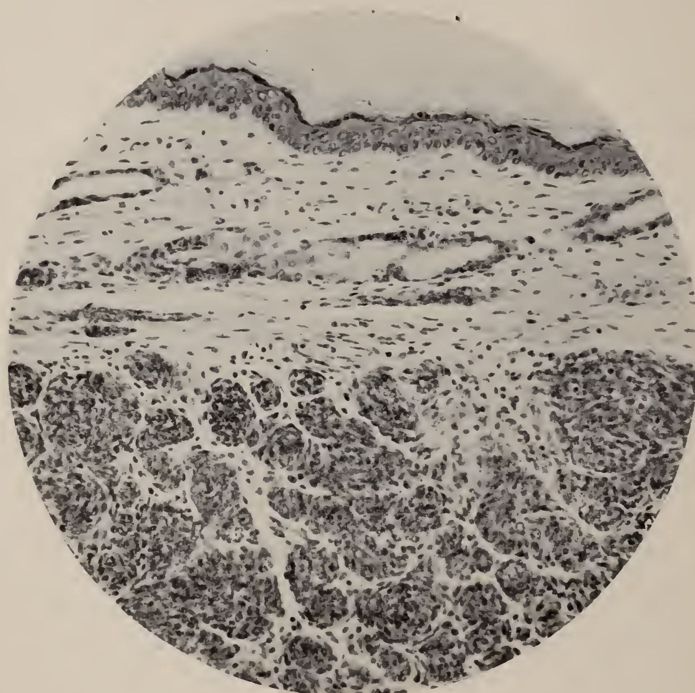

Fig. 1. Basal-cell epithelioma of rat (primary tumor). × 200

The interalveolar connective tissue showed marked proliferation and in places resembled sarcoma. As may be seen in the photomicrographs there was an unusual amount of lymphocytic infiltration about the tumor alveoli.

Seven weeks after inoculation, a few nodules were palpable in the 204 rats, and after nine weeks eighteen nodules in all were present. These increased in size with extreme slowness and six

of them reached dimensions sufficient for transplantation into a second generation. The largest was about 1 by 1.5 cm. and required 240 days for its growth.

Transplantations into a second generation were as follows:

1. Tumor 0.9 by 1 cm. 61 days post inoculation into 108 rats.
2. Tumor 0.8 by 1 cm. 62 days post inoculation into 120 rats.

FIG. 2. Basal-cell epithelioma of rat (primary tumor). × 200

3. Tumor (transplant into original tumor-bearing animal) 1 by 1.3 cm. in diameter 111 days after inoculation into 48 rats.
4. Tumor 1 by 1.3 cm. 119 days after inoculation into 54 rats.
5. Tumor 1.2 by 1.3 cm. 135 days after inoculation into 60 rats.
6. Tumor 1 by 1.5 cm. 240 days after inoculation into 68 rats.

In the first five series of the second generation, comprising 390 animals, several minute nodules appeared, but these failed to grow. A difficulty was experienced in the high mortality of

the animals from intercurrent disease. In the sixth series of the second generation one reached a size suitable for transplantation. This was transplanted into 48 rats, but failed to grow. One fragment was inoculated into the original animal and grew rather rapidly to a size 1 by 1 cm., when unfortunately the animal died. The histology of this tumor was pecu-

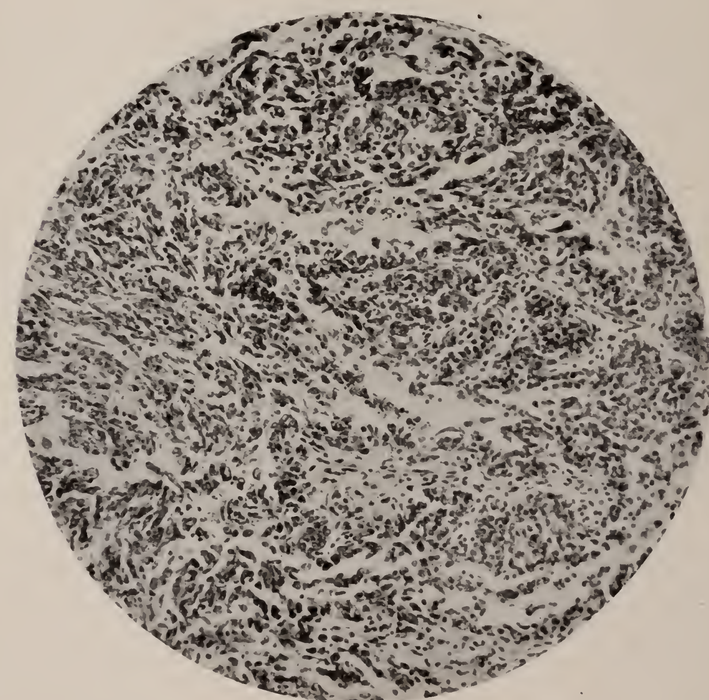

Fig. 3. Basal-cell epithelioma of rat (primary tumor), showing spindle-shaped epithelium. × 200

liar. While the characteristic basal cells occurred and the arrangement was typical in places of the spontaneous tumor, elsewhere there was an adenomatous arrangement and in one part a solidly alveolar type of tumor was found with dense masses of cells closely investing blood capillaries.

Microscopical sections of all tumors removed for transplantation showed the same general morphological characteristics as

the original growth. Further propagation of the tumor, owing to failure of the grafts, proved impossible and the tumor is now extinct.

Very interesting has been the observation of the grafts into the original animal, which lived for 377 days after the original tumor was removed. Into this animal two transplants from

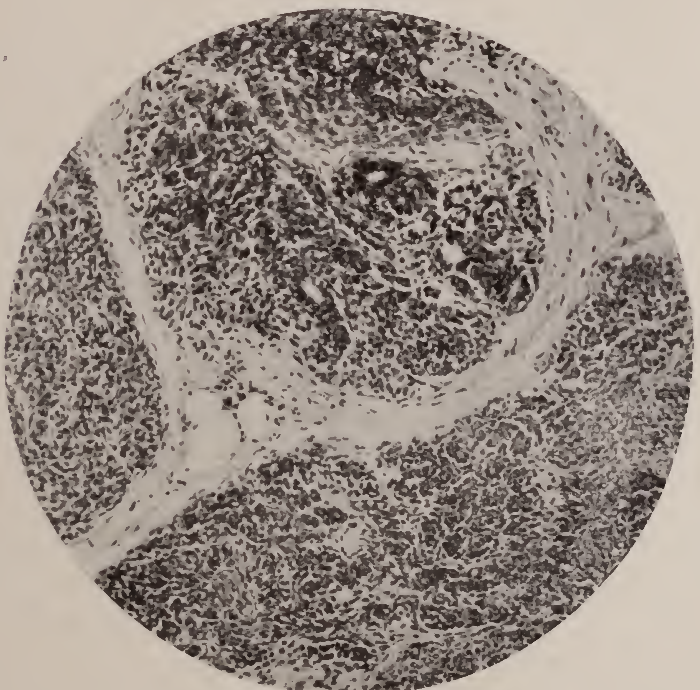

Fig. 4. Basal-cell epithelioma of rat (primary tumor), showing alveolar areas. × 200

the spontaneous tumor were made, one into the right axilla and a second into the right groin. Growth in these did not start for nine weeks, or later than the appearance of the nodules in some of the other rats of the first generation. This is contrary to the usual experience in autotransplantation. As soon as growth had begun, however, both tumors grew progressively and fairly rapidly, until at 111 days after inoculation the tumor

in the axilla had attained a size of 1 by 1.3 cm., when it was removed for transplantation. Two fragments of 0.003 gram were inoculated into the left axilla and left groin. These grew to a size of 0.5 by 0.5 cm. each and then completely receded. The tumor in the right groin remained stationary without increase or decrease of size after the removal of the axillary tumor. Three hundred and seventy-seven days after inoculation, the animal died and this tumor was removed for microscopical examination. It showed in places the same histology as the original tumor and elsewhere a peculiar adenomatous arrangement of the cells. It was free from degenerative changes and mitoses were very scarce.

Subsequently, two grafts from a tumor of the second generation were transplanted on right and left sides beneath the skin of the back of the animal in which the tumor originated. Both of these after reaching the size of a split pea completely receded. Failure to grow was not referable to infection of the tumor for it grew in other animals.

If we attempt to analyze these fluctuations of growth energy in the original animal we meet with some very interesting questions. If the primary growth and subsequent recession of the four grafts that underwent resolution be regarded as the development of immunity in the animal, why was not the tumor in the right groin likewise absorbed? Furthermore, the development of any immunity whatsoever to its own growth by a spontaneous tumor animal has been thought not to occur (1) and certainly ordinary immunizing injections of homologous tissue fail completely to cause it. Yet if we do not regard the recession of four grafts in an animal as the development of a species of immunity, how is it to be interpreted? The phenomenon of the recession of one spontaneous tumor while others continue to grow has been observed in rare instances before; thus Haaland (2) records an instance of a tumor in one groin receding in the animal in which it arose while an axillary tumor of the same variety continued to grow. He records also an instance of complete temporary recession of a spontaneous tumor of the vulva. Both these phenomena are, however, conspicuously rare.

A second feature worthy of note is the remarkable length of time (266 days) which the graft in the right groin of the original host remained dormant without increase or decrease of size. This is most unusual. This nicely adjusted balance which permits cancer cells to live so long in a host and yet not perceptibly multiply is more characteristic of the benign tumors, which until quite recently have not been successfully transplanted, or of lymph-node metastases, which in man have been recorded as lying dormant for ten years or more.

Another question which is aroused by the failure of the four grafts in the original animal is, why cells which are native to one animal should first grow in another animal and then on return to the original host fail to grow? These things are contrary to the customary experience in autologous transplantation and merit further research.

This tumor, as far as can be ascertained, is the only basal-cell epithelioma that has been found in an animal. Its sluggish growth rate and ability to lie dormant for long periods is quite analogous to the behavior of the same type of tumor in man.

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A STUDY OF FOUR CASES OF BEGINNING SQUAMOUS-CELL CARCINOMA OF CORNIFYING TYPE

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INTRODUCTION

The following pages are the result of a study of cases of very early and actually beginning carcinomata of the skin. In an earlier paper (1), I discussed in some detail von Hansemann's theory of anaplasia, and tried to indicate how naturally and easily that theory develops, in the light of present knowledge of the laws of inheritance, into one which regards the cancer cell as a product of somatic mutation. So simple and direct, indeed, is that development, that the difference is largely the difference between the terminology of thirty years ago and that of today. It follows of course that the present point of view contains little that is new. The anaplastic cell of von Hansemann becomes quite naturally the new race of cells of Hauser; so that I am by no means the first to apply either the concept or the term, mutation, to cancer genesis. But the application to the problem of the remarkable observations of Morgan and his associates gave me, I felt, such added understanding that it seemed worth while publishing it. The present paper attempts to approach the problem from the same viewpoint as the earlier one but to present it in a more objective, less abstract form. Since it is largely based on the same bibliography as the preceding paper, it has seemed unnecessary to repeat that given there.

The present study embraces seven cases about equally divided between the cornifying and the non-cornifying types. The non-cornifying tumors will be discussed under a distinctive title.

Ribbert (2) bases the distinction between the two forms on the mode of growth.

> In all cases where infiltration occurs by means of continuous elongation of the epithelial papillæ or processes, a cornifying carcinoma is produced, ... but when on the other hand entirely new-formed, independent buds invade the cellular connective tissue, growing from the basal layer of the epidermis or from the lateral aspect of the epithelium of the hair follicles or sebaceous glands, and especially from the lower epithelial layers, which have been converted into light staining, large cells, then a non-cornifying carcinoma usually develops.

I have made use of this criterion as far as it would serve. But as will become evident, it has not always served, since the type of growth does not always coincide with one or the other of these categories. On the whole I have felt that I could more safely rely on the following points.

1. *a*. Cornifying carcinomata, so far as my series is a guide, arise in verrucous keratoses.

b. Non-cornifying carcinomata, on the other hand, arise from areas where there is no verrucous thickening; often indeed, the epidermis is sunk below the level of the normal skin, apparently because of changes in the corium. The cases of doubtful classification in my series are of the first type, *a*, and I am by no means sure that the above furnishes a reliable index.

2. A more satisfactory guide is furnished by the character of the malignant cell. In the non-cornifying tumors, this very quickly settles into its established type, as a small, more or less spindle-shaped cell with deeply staining nucleus and scanty protoplasm; whereas in the cornifying tumor, the cells remain for a longer time at least, with larger, rounder, more vesicular nuclei and more abundant and more opaque protoplasm. Where the formation of pearls is early and abundant, the diagnosis is of course settled at once.

To save space I shall describe here briefly, once for all, certain degenerations which occur quite commonly in the connective tissue, especially in the cornifying cases. These changes are described in detail by Unna (3) as senile degenerations, occurring

regularly in the skin of all old people (50 years or more), especially of the face. The following is a much shortened description from Unna.

1. *Elacin.* Constant in the middle and deeper layers of the cutis; single and in bundles, broad, cylindrical, and wavy fibers which look deceptively like ordinary elastic fibers, but stain with basic dyes. The same fibers stain with specific elastic fiber stains, though somewhat less intensely than normal.

2. "*Blue areas.*" Immediately under the basement membrane in and around the areas in which elastic tissue is thickened and apparently increased, collections of basophilic collagen fibers which swell, lose their sharpness of contour, and break up into clumps and granules of varying size.

Unna's collacin and collastin degenerations I have not seen, probably because I have not looked for them in properly prepared sections. The blue areas in my preparations stain more or less deeply with hematoxylin, depending on the length of time the sections are left in the stain and the degree of subsequent differentiation (in acid alcohol). If the latter is pushed far enough the hematoxylin is entirely removed, but the areas resist subsequent staining with eosin. The same areas stain evenly with elastic fiber stains (I used Verhoeff's). I assume that these areas also contain collacin, which does not appear in the exact form described by Unna, because Verhoeff's stain is less precise than the orcein method employed by him. The elacin degeneration and the "blue areas" are mentioned only because they constitute a striking feature of several of the cases. The "blue areas" in particular seem to be identical with the so-called "precancerous condition of the connective tissue" seen, sometimes in conjunction with myxomatous degeneration, about the hair follicles in carcinoma of the skin, or about groups of gland acini in the breast. It is apparent that their presence is merely incidental. Similarly with regard to other and more delicate senile changes in the skin, such as thinning of the epidermis, with local thickening and pigmentation of the horny layer, shortening and disappearance of the epithelial papillae, changes in the hair follicles, depressions or dimples in the surface of the

skin, etc. All these are incidental to old age and will be mentioned, if at all, only incidentally. Of greater interest to the present discussion are the foci of leucocytic infiltration often found in the cutis, according to Neumann (4). Their frequent presence in the senile skin argues strongly against their having any such significant relation to cancer genesis as is assigned to them by Ribbert.

One more word of introductory explanation seems necessary to guard against a possible charge, through misunderstanding, of serious and obvious blundering on my part. I shall have frequent occasion to describe varying stages of malignant growth as occurring side by side in the same area. Usually the more advanced stage is found near the center of the area involved, with younger growths scattered about in the neighborhood. These younger growths I do not consider to be metatases, for what, I trust, will be regarded as obvious reasons. For equally obvious reasons they are not to be regarded as indicating a spreading infection, nor as appositional growth, i.e., secondary "infection" and conversion into carcinoma cells of neighboring normal epithelium by the cells of the earlier and more advanced growth. We must assume, I think, that mutative changes can occur only during the process of mitosis. This being the case, the presence in a given area of those unknown conditions favorable to cancerous mutation is not enough, taken alone, to produce a cancer. Their effective functioning must wait upon the occurrence of mitosis. Doubtless also many mitoses occur in the area, without falling victim to the disturbing influence; and since it is hardly conceivable that any considerable number of mitoses should occur simultaneously throughout the area involved, and since, even if this should happen, it by no means follows that the rate of growth in all the cancers thus started will be alike, nothing is more naturally to be expected than that any area of multicentric growth will exhibit many independent growths of various ages, or at least, stages of progress.

I shall describe the cases in the order of their stage of development, beginning with the youngest.

CASE 1. CORNIFYING CARCINOMA ARISING MULTICENTRICALLY FROM BASAL AND PRICKLE CELL LAYERS OF EPIDERMIS AND HAIR FOLLICLE

This was one of two senile warts removed in 1909 by Dr. C. B. Lyman from the back of a man about seventy years old. Over an area of about 6 mm. in diameter there is a moderate rough keratotic thickening rising 1 to 2 mm. above the level of the normal skin (fig. 1). In the region surrounding this elevation, areas of the germinal layer of varying size contain a finely granular dark pigment. These cease a short distance from the edge of the verruca. Under the basement membrane, and extending the entire length of the section, except immediately below the verruca proper, is a succession of irregularly round or oval blue areas (fig. 1, *a*). The basement membrane is a delicate layer of collagen fibrils, varying somewhat in thickness. Even at the extreme ends of the sections, it exhibits a tendency to become hyalin and lose its nuclei. As the center is approached this tendency increases, the fibrils swell and break up into coarse lumps which still retain their normal color reaction to eosin; the nuclei disappear almost completely and the whole structure is finally replaced by a hyalin vacuolated mass suggesting a coagulum. But the process is not continuous and areas of more normal appearing basement membrane occur here and there. At the edge of the verruca where budding of the epithelium is beginning, the epithelial cells of the basal layer abut on a very narrow open cleft, beneath which is a delicate stroma of degenerated, partly disintegrated collagen fibers (fig. 2, *1*). There is a scanty small round-cell infiltration about the blue areas and between the latter and the epidermis; also and more abundant about the hair follicles, sebaceous glands, and the capillaries supplying these.

The epidermis beneath the parakeratotic area is thickened and exhibits many irregularly shaped buds extending in various directions into the corium. Some of these are found at some little depth in the corium, cut in cross section and apparently free and detached from the epithelium. The corium here is only slightly infiltrated with small round cells, and this infil-

tration is practically confined to the zone of connective tissue immediately surrounding the epithelial buds above mentioned. I shall describe some of these buds in greater detail.

The area shown in figure 2 is a higher magnification of the area shown at the left in figure 1, marked *b*. The bud at *A* consists entirely of cells resembling somewhat those of a non-cornifying carcinoma, but with more abundant and pink staining protoplasm. The nuclei are round or oval, dark, and not regularly arranged with their long axes at right angles to the plane of the basement membrane, which as already noted, is partly deficient. The oblique line marks approximately the line of separation between visibly altered cells, and those apparently still normal. The still intact basal layer of cells can be seen extending across the base of the bud about one half of the distance from the left to the right, where the line *A* terminates. The bud itself seems to arise from that portion of the basal layer to the right of this point. This represents a very early stage of visible change in the cells. Indeed taken by itself, it could not of course be recognized as a step in the malignant process.

The larger bud at *B* exhibits a somewhat later stage. At the point where the basal cells turn inward over the bud (*1*), the basement membrane suddenly disappears. From this point leftward the basal cells change progressively in character. Here the crooked line drawn through the bud marks the approximate line of demarcation between the proliferating basal cells above and to the left (in the photograph) and the prickle cells below and to the right. The former are distinctly plumper, deeper staining, and more irregularly arranged than normally. They show a distinct tendency to group themselves in spherical masses or lobules, similar to the groups sometimes seen in mucous membranes at the beginning of papilla formation. At *3*, they are beginning to send delicate protoplasmic bridges suggestive of pseudopodia, into the degenerated, partly disintegrated connective tissue. Significant changes however are not confined to the basal cells. The prickle cells are large, and less flattened and their nuclei darker and less spindle-shaped than is proper to spindle cells of the corresponding level of normal epidermis. At

three points (marked *2*) there are small groups of darker cells concentrically arranged, corresponding to an early stage of pearl formation, or to cell groups isolated in situ and growing independently. Considering that this particular bud is at the extreme edge of the verruca, and the absence of pearls in the verruca itself, the formation of pearls here is not without significance. More striking evidence of involvement of the prickle-cell layer will be cited shortly.

Figure 3 is a higher magnification of the point marked *c* in figure 1. It shows part of a cancer bud of considerable size. This is connected with the epidermis covering the verruca above by a strand of elongated prickle cells which passes through the basal layer and can be followed almost to the horny layer (*a*). The cells composing it are sharply differentiated from their neighbors within the prickle-cell layer by their lighter color, more elongated shape, and sharp difference in the angle at which their long axes are placed. The whole picture is curiously suggestive of a crowd streaming through narrow doors. By no possibility can it be interpreted as due to secondary adhesion of malignant to normal epithelium, or otherwise than as indicating the origin of the cancerous growth from the prickle-cell layer.

Figure 4 is a higher magnification of the area marked *d* in figure 1. The dark mass of epithelium in the center is a hair follicle. At either side (probably indeed encircling it in the tissue), are unmistakable cancer buds springing from the point of juncture of the hair follicle with the epidermis and extending slightly further down the length of the follicle on the left than on the right. It is reproduced partly because it seems to offer further incontrovertible evidence of the share taken by the prickle cells in the production of the cancer (observe especially the large deep staining unmistakably mutated (anaplastic) prickle cells at the base of the bud on the left); and partly because of the relation stated by Mallory (5) to exist between the hair follicles and non-cornifying carcinoma. This carcinoma, which belongs, I think, to the cornifying type, arises from the hair follicles as well as from the epidermis. Compare Ribbert (2, p. 155 and fig. 119) where a like origin of a non-cornifying carci-

noma is described. Near the center of the picture, and just to the left of the hair follicle is a group of carcinoma cells which was probably continuous with the downgrowth at the right of the hair follicle.

At several points (fig. 1, *e* and *f*), more advanced stages of growth can be seen—fairly deep-seated buds unmistakably malignant in character, some with large pearls in various stages of formation. In spite of the fact that the cell buds do not grow straight into the tissue, but send out many secondary buds (fig. 3, for example) very early, and in spite of the fact that the cells of the rather large group shown in the center of figure 4 are rather small and deep staining, i.e., of the non-cornifying type, I am convinced that this case should be classified as cornifying. Possibly it represents one of the mixed type described by Krompecher.

The outstanding features of this case are:

1. The striking absence of inflammatory reaction except in the sharply circumscribed zone immediately surrounding some of the older and more advanced buds, and about certain hair follicles and sebaceous glands.

2. The very early stage of the growth in many areas. Many of these, taken by themselves, could not be regarded as indicating malignancy. But the location of these growths inside the margin of the verruca excludes ordinary inflammatory proliferation of the epithelium with the single possible exception of the small bud first described and shown in figure 2.

3. The multicentric growth, involving both the basal cells and the middle layers of the epidermis.

4. Origin from hair follicles as well as from epidermis.

CASE 2. CORNIFYING CARCINOMA ARISING DIFFUSELY FROM GERMINAL LAYER OF EPIDERMIS

This is a keratotic wart (fig. 5) removed by Dr. O. M. Gilbert from the scalp of a woman about fifty years old. It is approximately 5 mm. in diameter and rises abruptly above the surface of the surrounding skin. In general it resembles case 1 closely. The striking points of difference may be summarized as follows:

1. Blue areas occur as in the preceding case, and in addition small areas may be found in the markedly inflamed corium underlying the verruca.

2. Elacin degeneration of the elastic fibers is present to a marked degree among the elastic fibers in the deeper layers of the cutis.

3. The inflammatory reaction is slight beyond the margin of the verruca as in case 1, but beneath the verruca is much more intense and widespread. In the area surrounding the verruca the blood- and lymph-vessels are distended, their endothelium is somewhat plumper than normal, and the vessels are accompanied by a scanty accumulation of small round cells. Under the verruca the blood capillaries contain an occasional hyalin thrombus and are frequently filled with leucocytes, and the surrounding tissue is quite densely infiltrated with wandering cells, among which polymorphonuclear cells, while not the predominating type, constitute a striking feature. The inflammation extends almost to the depth of the lower terminations of the sweat glands.

4. *The epidermal cells.* The cells of the granular layer are here and there, especially near the outer border of the verruca, infiltrated with leucocytes, chiefly polymorphonuclear cells. The cells of the prickle layer are somewhat large and plump, i.e., less flattened than normal. At one point only (fig. 5, *a*) I have found a bud of such size that an offhand diagnosis of carcinoma would be possible. It extends deep into the corium, in the form of an inverted Y, and consists of rather large spindle-shaped cells, whose long axis is directed for the most part inward (or downward); the nuclei are rather large, very pale, vesicular, round, oval, or spindle-shaped, with one or two large, deep staining nucleoli (fig. 6). Protoplasmic bridges between the cells can be made out in a few places. The contour of the bud is somewhat irregular. Over part of its extent it is bounded by a somewhat diffuse pseudo-basal layer consisting of cells like the rest except that they stain more deeply. The tips of the bud are somewhat infiltrated by round cells and become lost in the fairly dense inflammatory infiltration about them.

The precise center of origin of the process from the overlying epithelium is not in the sections. It lay, I take it, too nearly parallel with the plane of the section to show, or else it was discarded in some of the sections torn in cutting; but a connection, clearly not secondary, can be seen with the tip of the overlying epithelial papilla, where the basal cells are everted over the beginning of the bud and cover its base for a certain distance (fig. 6, *a*). The prickle cells continuous with the process inside the everted basal-cell layer show no abnormality in size, shape, or staining reaction, and I therefore infer are taking no essential part in the growth.

There is no pearl formation anywhere but the type of cell in this bud settles the diagnosis as cornifying carcinoma.

At many places the basal-cell layer over considerable stretches shows a marked heaping up. The earliest stages of this change cannot, of course, be recognized with certainty. Apparently the basal cells first become somewhat larger and darker, so that they stand out with more and more distinctness and tend to lie flat side to the basement membrane (fig. 7). Later they increase to many layers in thickness, become more and more disorderly in their arrangement and tend to separate from one another (fig. 8). In this condition they are readily distinguished from the familiar artefact, due to oblique cutting. Apparently at any stage actual budding may occur (figs. 8 and 9). In some cases at least, such budding takes place by the growth inward and laterally of a small group of basal cells, in such a way as to carry the basal cells on either side with it for a certain distance (fig. 9). In this way it produces perhaps the inverted basal-cell layer described above.

Figure 10 shows early growth from the upper portion of a hair follicle. The only question which can arise, I think, is whether the growth here shown is due to changes in the follicular epithelium proper or to extensions along the follicle of growth arising in the neighboring epidermis. The absence of any line of demarcation between normal and proliferating cells settles the question in favor of the former interpretation.

Figure 11 is the epidermal layer from a large verruca, for comparison. It will be observed that here too a fairly marked inflammation is present. The orderly disposition of the cells forms a marked contrast with the earlier figures. I have frankly accepted, as belonging to the cancerous process, changes in the cells which, taken by themselves, would not warrant that diagnosis. This is in accordance with my conviction, as explained in greater detail later, that a disease (typhoid, for example), begins at the moment the disease process is set in motion. In the present case stages of the disease are present which are clearly recognizable as such: there are other, and earlier stages which, standing alone, could not be so recognized. But the relation of the latter to the former can be traced. It is clear that they belong together.

The case is, then, one of cornifying carcinoma beginning rather diffusely in the basal layer of epidermis and hair follicles and without appreciable involvement of the more superficial layers.

CASE 3. CORNIFYING CARCINOMA ARISING DIFFUSELY FROM THE BASAL-CELL LAYER ONLY OF A VERRUCA

This is a verruca measuring 4 mm. in diameter, removed from the helix of the ear of a man, fifty-five years old, by Dr. C. E. Giffin. Its top is covered by a thick, rough, laminated mass of keratinized epithelium which is attached at its base to epithelium covering a fairly deep and sharp depression in the surface of the skin (fig. 12). The lower (inner) level of the epithelium over this depression roughly corresponds to the level of the corium containing the papillae of the hair follicles of the surrounding skin. But the skin of this region rises slightly to meet the sides of the verruca, and the epithelial papillae become gradually longer and thicker in the same direction. At the edges (or ends) the basal layer is pigmented in spots, which disappear toward the center. At the edges also the epithelium rests upon a sharply defined, firm basement membrane of connective tissue, whose fibers run parallel to the surface of the epithelium which they support. Toward the center again these

fibers swell, lose many of their nuclei, become hyalin, then break up into lumps and coarse granules, and, finally, at or near the edge of the verruca disappear here and there, leaving a narrow cleft containing granular detritus. Across this cleft extend (in some sections) delicate epithelial processes which increase in size and number, as one passes inward along the first large epithelial papillae belonging to the verruca, as in case 1 suggesting pseudopodia.

Under the basement membrane the collagen fibers of the cutis are converted into a dirty blue staining, granular and lumpy mass, almost entirely devoid of nuclei. The line of incision for the operation passes through this level, so that the condition of the deeper layers of the cutis cannot be determined. The region containing blue areas ceases abruptly at the edge of the verucca proper, the greatly elongated epithelial papillæ of which extend into the cutis far beyond the level at which the degeneration is found on either side.

One of the most striking peculiarities of the case is the almost complete absence of wandering cells. A very few minute foci may be found, partly near the edges of the sections, in connection with the blue areas, partly in the region of the verruca, gathered about buds of proliferating epithelium.

Cursory examination of the sections creates the impression that the basal layer underlying the verruca proper has disappeared, and that proliferation is taking place from the prickle-cell layer only, by means of long branching and anastomosing streamers of cells, extending into the corium. More careful study shows that the earliest stages of growth take place by a process similar to, but not identical with, the bleb-like proliferation which characterizes case 4. Here however, the change obviously involves the basal cells primarily, rather than the prickle cells as in case 4. In the present case the basal cells, as the first visible step in the process, become larger and more deeply staining than normal, become separated from the overlying prickle-cell layer, and bulge inward toward the connective tissue. In this condition they are often still arranged regularly side by side, and being taller than normal, they closely resemble

a columnar-cell mucous membrane (fig. 13). At the next stage the basal layer is found thrown into folds whose tips, at first pointing inward, fold and branch in various directions in the underlying connective tissue (fig. 14), and grow into the capillaries and lymph-vessels. Here they become arranged on the walls about a central lumen, replacing the endothelium. In some of these, structures resembling red cells, really spherical masses of keratin, may be found. Thus here and there the growth presents a resemblance not too remote if allowance be made for the fact of malignancy, to the so-called parotid mixed tumor (fig. 15). The cells now branching and anastomosing along the paths furnished by the lymph-channels, the picture is finally presented of a tangled mass of branching lymph or blood channels formed of epithelial cells. The cells forming these channels undergo keratinization, and the lumen thus becomes filled with a material resembling hyalin collagen, but which is clearly of epithelial origin. This change increases to the abundant formation of large and characteristic pearls. In some of the latter protoplastic bridges still exist between the cells.

Finally, the cells lining these gland-like channels proliferate, both inward toward the lumen and outward toward the surrounding connective tissue. This converts the gland-like structure into solid cords of cells which still anastomose with one another but gradually expand and obliterate the intervening connective tissue.

In the meantime the cells of the altered basal layer have proliferated outward (toward the prickle-cell layer), as well as inward (toward the corium) (fig. 13). Apparently the cells thus formed apply themselves closely to the pre-existing prickle-cell layer and become indistinguishable from these. It thus becomes practically impossible to determine what share, if any, the prickle-cell layer has contributed to the formation of the cancer. But it is, I think, clear that the presence of keratin and protoplasmic bridges in the tumor cells, as above described, cannot be taken as in the least proving the origin of the tumor from the prickle-cell layer, as is evident also from the genetic relationship of the two layers.

As the operation did not remove quite all of the growth, it is impossible to determine just how far its roots extend. It is perhaps the most advanced of the series, and in spite of the fact that its processes do not grow straight downward without branching, and in spite of the small size of its cells, the abundant pearl formation places it indisputably in the cornifying group. Here and there small aggregates of cells of the non-cornifying type are encountered. Several of these, traced through the series, proved to be parts of hair follicles, and I am quite sure that none of them represent neoplastic growth.

CASE 4. CORNIFYING CARCINOMA ARISING DIFFUSELY FROM PRICKLE-CELL LAYER ONLY

A verruca removed by Dr. C. E. Giffin from the face of a patient about sixty-seven years old. The keratotic area is 5 mm. in diameter and arises some 3 mm. above the surface of the surrounding epithelium.

In section, changes similar to those already described are found in the basement membrane. Just under the basement membrane are a few scattered foci of leucocytic infiltration among which a rather large number of eosinophiles form a striking feature. Still deeper is a broad zone in which the collagen fibers have undergone the basophilic change already described. This zone extends almost unbrokenly beneath the base of the verruca from side to side, answering perhaps to the fact that the base of the latter is less depressed below the level of the surrounding epidermis than in the other cases. Beneath the blue areas are small collections of elastic fibers which have undergone elacin degeneration, but this change is less intense and less widespread than in the other cases.

Turning to the epithelium, we encounter what at first sight appears to be two separate and distinct modes of cancerous growth existing side by side. First, the epithelium in the right half of figure 16 is increased to five or six times its normal thickness. But this thickness is due only in part to a diffuse plumping up of the epidermal layer. Most of it is apparently due to the elongation of papillae which also spread laterally and unite with

one another more or less completely, along their edges or lateral aspects. This will be more evident from figure 17 which is from the same area but at a different level, and in somewhat higher magnification. Here there is no granular layer. The outermost cells of the prickle-cell layer are very pale and apparently hydropic, as indicated by the many large clear spaces separated by mere shreds of cells. Many nuclei are represented by shadows only, and many seem to have disappeared completely. At a certain level the cells show a distinct tendency to change the direction of their long axes, so as to lie at right angles to the surface (*A*). From this point inward they grow somewhat irregularly, so that sometimes a papilla is narrower at its base than at its tip (*B*), due to pressure at its sides, and papillae apparently fuse when this pressure becomes great enough (*C*). As the cells progress inward they become also more and more atypical. At the points marked *D* this departure from type is well marked and the growth is distinctly infiltrative.

The surprising thing is that all this takes place within a basal layer which is well preserved, and for the most part normally arranged. This basal layer rests upon a basement membrane whose delicate fibrils are widely separated by fluids, and in part are no longer in direct contact with the basal cells. Practically nowhere is the membrane completely lacking.

In other sections from this same region, unmistakable malignant budding is in progress. The character of this budding will be described in detail in connection with the discussion of the left half of the field shown in figure 16, and is therefore omitted here.

Mitotic figures are abundant in the zone of thickened epidermis above described, both in the lower layers of prickle cells (fig. 18) and in the definitely cancerous buds above mentioned. Many of these are markedly hypochromatic in von Hansemann's sense. In one field, for example, I found two monasters in a non-malignant area, one of which contains something like thirteen chromosomes, the other apparently nine; near these, lying free in the cavity of a small gland-like malignant bud, a degenerating cell containing five. Mitoses cannot of course be

shown entire in photographs since the lens lacks depth of focus to include the whole figure. I am omitting a detailed description of this phase of the question at this time, partly because it would extend the present paper unduly and partly in the hope that more accurate observation will become possible when I can obtain a binocular microscope.

The apparently second mode of growth is encountered at various levels in the region corresponding to the left half of figure 16, and, at other levels than that shown in the figure, in the region corresponding to the right half of the same figure.

The earliest stage I have been able to identify with any certainty, is shown in figures 19 and 20, from the area just within the edge of the verruca. The whole epidermal layer is thickened, because (aside from increase in the horny layer) of an increase in the prickle cells. This thickening begins rather sharply at the exact edge of the verruca and then increases gradually toward the center. At about the middle of the prickle-cell layer the cells quite abruptly turn their long axes perpendicular to the surface. Here and there small groups of cells, somewhat darker than their neighbors, are found lying free in small pockets in the epidermis. The outermost row of cells of these groups is closely applied to the neighboring prickle cells and from some of them extensions of proliferating cells can be traced downward toward the basal layer. At the same time the cells of the basal layer become taller than normal and though still quite regularly disposed, bulge inward so as to present an irregular, wavy, lobulated outline. The basement membrane has entirely disappeared, its place being taken by a rather broad cleft partly filled by a vacuolated hyalin coagulum. In other areas vacuoles, really minute blebs, appear among the prickle cells just above the basal layer. These, enlarging, coalesce (fig. 21). It is perhaps significant that although many mitotic figures, some atypical, are to be found as already mentioned among the prickle cells, I have not found a single one in the basal layer.

Later stages are seen in low magnification in figure 22 which is the left half of figure 16, though from another slide; and in higher magnifications in figures 23 and 24. In figure 22 the

character of the inflammatory reaction present is fairly well shown and the blue areas are also seen (*A*). At *B*, *C*, *D* are three small cancer buds; *D* is shown again in figure 23, *C* in figure 24 and *B* in figure 25. In figure 23, the preservation of the basal layer with bulging and lobulation is well shown. In figure 24 the changed direction of growth of the prickle cells, as far out as the granular layer, is very evident. As the bud enlarges, the basal layer remains intact and tends to arrange itself in the form of gland acini, as is already suggested in figures 23 and 24, and more clearly indicated in figure 25. This feature is a very puzzling one. I have never seen such structures in a fully developed carcinoma of the skin. Figure 26 seems to indicate that this gland-like arrangement soon disappears, the cavity filling with cells, sometimes very atypical, which mingle with the outer gland-like cells derived from the basal layer. In these proliferating prickle cells the early stages of an abundant pearl formation may be met with. The case corresponds, therefore, fairly well to the criteria laid down for cornifying carcinoma. Partly for this reason and partly because of the general character of the cells, I have become convinced that it belongs in that class.

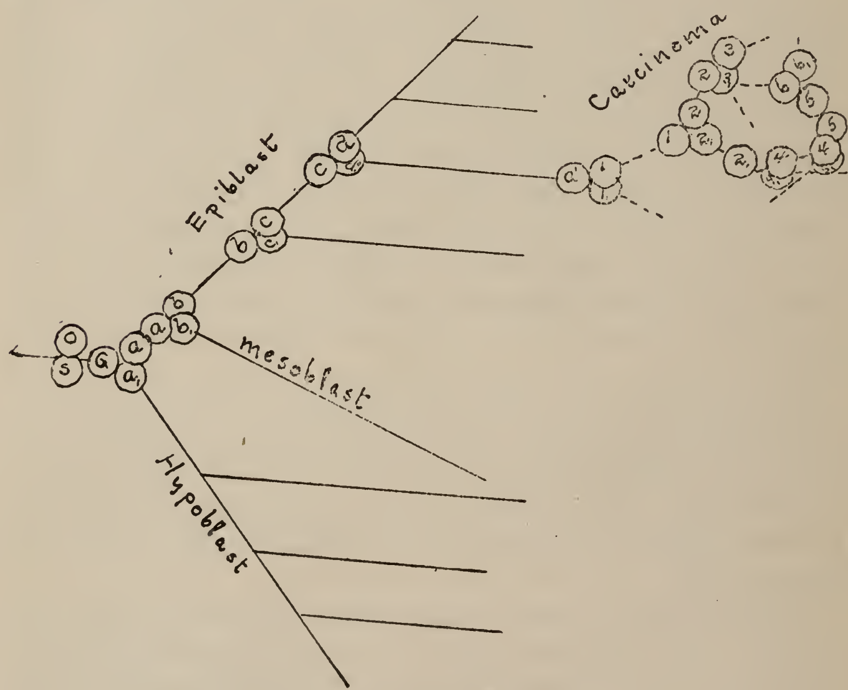
Returning now for a moment to figure 16 and the first mode of growth, it is apparent that the two modes are really one—a conclusion fully established by the fact that in some of the sections the difference between the two halves of figure 16 disappears and the epithelium everywhere presents the characters shown in the right half.

Finally, figure 27 shows a hair follicle clearly involved in the growth. That this is not due to secondary extension along the follicle is shown by the absence of any line of demarcation between the normal and cancerous cells.

The case is then one of cornifying carcinoma arising diffusely over a wide area of epidermis and also from hair follicles, and involving the prickle cells only. It is characterized especially by the growth of these cells within an intact basal layer, which at first enlarges to make room for the cancer cells, but later apparently disappears.

THEORETICAL CONSIDERATIONS

Whoever enters the field of speculation, knowingly ventures on very thin ice, and takes, as it were, his life in his hand. But I believe that in dealing with this subject, a record of observed facts can have but little value without an honest effort to understand and interpret those facts. It is not enough in other words merely to observe the apple falling. We must be not afraid to guess, since the accuracy of the guess measures the value of the whole effort.



Text Fig. 1

First, then, what is a cancer cell? This point I have recently discussed at some length and reached the conclusion that a cancer cell is a mutated somatic cell. It is not an embryonic cell, a cell presenting some sort of permanent, local infantilism. On the contrary, the cancer cell is a new cell, differing in its biologic properties from any cell at any time normally present in the body. Perhaps the point can more clearly be apprehended

by reference to the accompanying diagram (text figure 1). Let the circles at the left represent the sexual cells which unite to form the germ cell. The germ cell multiplies and separates into two layers, epiblast and hypoblast (*a* and *a'*). In man the mesoblast develops from the former, as indicated (*b*). From the three layers thus formed the various organs and tissues develop.

Each new stage in development, indicated by a line branching off from the main trunk, is the result of an asymmetrical division; i.e., a cell, represented by a circle in the diagram, divides mitotically so as to produce daughter cells having unlike biologic properties, from *b*, *c* and *c'*; from *c*, *d* and *d'*, etc. One of these gives rise to the new tissue, the other continues in the same general direction of ontogenesis as the mother cell, though it also differs from the mother cell, to the same extent but in the opposite direction, from its twin. If we assume, as I think we must, that every property of the cell depends upon the presence within it of definite structures (genes or factors), it is difficult to conceive of any other way by which one sort of cell might arise from a different sort. When development is complete, this process of differentiation comes to an end, except as it is renewed from time to time, within narrowly circumscribed limits, in the processes of repair.

If the above is true it follows so obviously, I think, as to be axiomatic that a tumor cannot by any possibility be produced through the reversion of a mature cell to an embryonic stage of development. For no cell can go backward on its normal path of ontogenesis farther than the point at which it left the parent stem, since it cannot regain, out of nothing, the properties it lost in the unequal mitosis which gave it its origin. That is, *d'* cannot become *c*, since it cannot regain the properties originally lost to *d*. But at such a point of reversion the cell is nothing more than the normal embryonic cell of its kind—a fibroblast, for example, or a young, growing, but otherwise normal, epithelial cell. Certainly it is not a tumor cell.[1]

[1] It is not to be assumed that the separation of functions by unequal mitosis is absolutely complete; von Hansemann assumed that the new cell contains chief plasmae (or genes) (*Hauptplasmen*), for which I now prefer the term kinetic

If then, a tumor (a carcinoma, to take an extreme case) cannot arise by following its normal path of development in the reverse direction, it follows, since the carcinoma arises from an epithelial cell, that the departure from type must be in some other direction. That direction may be any you please, except the one. This conception of the process is illustrated in the diagram by the broken lines starting from the line which represents the epidermis. Anaplasia is this departure from type in a new direction (see my previous article).[2] "Anaplasia begins where normal ontogenesis leaves off" (von Hansemann). Moreover, since the direction of deviation from type is not constant (see above, cases 3 and 4), and since in the growing carcinoma ever new processes of deviation (i.e., new mutations) are or at least may be taking place, it follows that when established, the tumor as a whole is not necessarily a single entity; but it may be a jumble of entities, as I have sought to represent in the diagram, in which each broken line stands for a special variety of cell.

Thus far my argument follows von Hansemann's theory of anaplasia. But my conception of the process differs from his at this point, and I shall, therefore, develop here in greater detail a point of view which was touched lightly in the article previously cited. Von Hansemann believed that the "anaplastic" (tumor) cells arise by a process of unequal mitosis, analogous to that by which normal development takes place but differing from the latter in that the inequality of division involves whole

genes; these are the actively functioning factors, and determine the normally expressed characters of the cells; and secondary genes (*Nebenplasmen*), which I now call latent genes. Under normal conditions these exercise no obvious influence on the life and form of the cell, but may do so under abnormal conditions. For example (I am not quoting von Hansemann now), they may determine the limits within which metaplasia can take place, and measure the capacity for reparative growth through reversion to the embryonic condition.

[2] In general I have avoided the use of the terms, "anaplasia" and "undifferentiation," in favor of the term, "mutation," not because the first two (particularly the first) are less serviceable than the last; but because the two former imply to most minds a process of reversion to an earlier, i. e., embryonic, state of existence; while the word, "mutation" leaves us free to imagine the cancer cell as one embarked on an entirely new career of *re*differentiation, the direction of which is anything you please, *except backward.*

chromosomes or perhaps groups of chromosomes. Of two such unequal cells the one which suffers a loss of chromosomes is "hypochromatic" and ultimately disappears. The other is "hyperchromatic" and constitutes the cancer cell.

Now while I by no means wish to deny the occurrence of asymmetry in von Hansemann's sense, I do not find it particularly helpful. For it is obvious that the hyperchromatic member of a pair has not, by virtue of such asymmetry alone, lost any factors or genes; on the contrary, it has gained those chromosomes and their contained genes, lost by the smaller cell. If we might assume that such an addition of chromosomes would cause a change in properties geometrically proportional to the extent of the addition, the case would be simple enough, for we could then suppose that the added genes had exalted certain characters by multiplying the number of identical genes present in the cell. But such an assumption is not justified, as I have shown in my earlier article. We may imagine if we choose that the addition of one or more chromosomes renders mutation easier and more likely to occur, by upsetting the smooth working of the mitotic process and by making what must be a really huge addition to the factors controlling the function and life of the cell. Their joint effect might find myriad expressions.

It is evident that I have enjoyed one decided advantage over readers of this essay, in that I have been able to select for illustration those features which seemed to me to favor most my own views. Doubtless also in spite of the best will in the world to the contrary, the selection of fields for discussion and illustration has been influenced by my personal attitude. On the other hand it is presumably not of prime importance whether my own explanations for the varied phenomena of growth observed are accurate to the last detail. I feel safe in saying that no one can possibly differ with me more earnestly or in more directions than I have differed with myself from time to time in the course of these studies. But it is important that in a series of four cases of carcinoma, of the same general type, no two are exactly alike in the details of origin and growth. There is no apparent reason for assuming that these differences are the

result of adaptation to differences of environment. On the contrary I believe that the whole theory of adaptation has been sadly overworked and that it is at least as rational to assume, for example, that the mole and the blind fish live in darkness because they are blind, as it is to assume that they are blind because they live in darkness. My own mind rests, more contentedly than on either of these notions, on the assumption that they live in darkness because they have lost the factor for heliotropism. My article, already cited, discusses this question at greater length.

I prefer, therefore, to regard these differences in behavior as proof of corresponding differences in the inner structure and life of the cell. The cells of a tumor, like the cells of the body, are subject to laws of growth imposed more from within than without. If we apply the law that "cells feed, but are not fed" (which like many laws is valid only within certain limits) we can see that a quantitative or qualitative deficiency or excess of food, for example, while it might cause various diseases of the cancer cell, could not in and of itself, affect the fundamental biologic laws controlling the cell, beyond contributing, so to speak, to the delinquency of the cell by serving as the occasion, but not the cause, of new mutations. Similarly with regard to pressure effects. Pressure might, to be sure, modify the external shape of the cell as a tight bandage modifies the shape of a foot. But the foot remains a foot, normal so far as the intrinsic laws of its existence are concerned. Moreover, such an effect is usually easy to recognize through its spacial relation to its cause, and the cancer cell is able to escape readily from such effects, by destroying the cells that press upon it. Granting the validity of these considerations we can, I think, see in the changing behavior of the cells definite ocular proof of changing constitution.

It seems to me, therefore, that mutation of the cell (whatever that may be), is the essential element in the origin of the cancer cell. Nor need we imagine that the change from a normal to a malignant cell necessarily takes place at a single step, as Minerva sprang full panopled from the head of Jove. There are plenty of facts to indicate, as von Hansemann among others long ago pointed out, that the biologic state of a growing cancer is any-

thing but stationary and that functions present in the parent tissue may become progressively changed or lost.

It is, of course, exactly this fact that renders the theory of mutation applicable to all tumors, whether benign or malignant, whether they arise from a cell rest or from an anlage normally intercalated in the tissues. An adenoma or a fibroma remains an adenoma or a fibroma until a further mutation or series of mutations converts it into a carcinoma or a sarcoma, respectively. An osteochondrofibroma, for example, may arise from a homogeneous tissue whenever that tissue retains as "latent genes" (suppressed by inhibiting factors in its mature state) the factors necessary to the production of the several sorts of tissue present in the tumor; the loss of one or other of the inhibiting factors in this or that area determining the growth of such areas in the corresponding direction. We are also afforded a clear insight into the otherwise puzzling fact that a tumor belonging to one embryonic layer can never become a tumor belonging to a different layer. If we regard the tumor cell as the result of undifferentiation backward along the path it followed in normal development, we must assume its ability to follow that path beyond the point where it separated from the cell that gave it origin. If it can do this, there is no great reason why it might not go still farther along the same path to and beyond the point where the embryonic layers formed, and thus become, from, for example, an epithelial cell, an earlier indifferent cell manifesting characters belonging to other layers. This we know never happens, and this becomes readily comprehensible when we regard the tumor cell as the product of re-differentiation along new paths, with characters dependent on the total effect of the factors present, which, however modified by mutation, cannot produce the characters of other embryonic layers, since none of these were present in the parent cell. Tumors containing elements of more than one layer can arise only from pluri- or totipotential cells, or from a cell rest isolated later, and containing cells belonging to each of the layers present in the tumor.

Indeed, the theory, whose acceptance no doubt demands an act of faith, is as flexible to serve varied purposes as is the side-chain theory in its particular domain.

The cancer cell, then, or, more broadly, the tumor cell may be defined as a cell arising by mutation from a normal (though perhaps displaced as a cell rest) somatic cell. So long as the new species of cell thus produced continues to grow without undergoing further mutation, the character of the tumor will remain constant; but when new mutations occur, from time to time and here and there, new sorts of tissue arise to correspond.

This leads me to protest against the familiar habit of describing or even defining this or that tumor as "tending to differentiate as" or as "seeking to produce" this or that parent tissue. If we accept the mutation theory, we must believe that a tumor is not a mere abortive effort to produce something else than itself. It is what it is by virtue of the properties inherent in its factors and can be properly regarded as growing only in obedience to its own laws of growth and "tending to differentiate as" just one thing, namely, itself. The familiar phraseology is really as absurd as it would be to speak of a pig, seeing its two-toed hoof, as tending to differentiate as a cow, of which ideal it falls short by its inability to grow horns and regurgitate its food.

Second, when then shall we say that a cancer has begun? It is apparent that a precise answer cannot be given, but I believe we can get much nearer a correct answer than that represented by the familiar view, that carcinoma exists only when the process of infiltrative growth has made it clearly recognizable under the microscope. Analogies are dangerous, but sometimes useful. When shall we say that tuberculosis has begun? When it is diagnosed? Clearly such a view is nonsense. Then, when if looked for, definite histologic evidence of it could be obtained? Clearly again there must in every case be a stage when changes exist so delicate that even the most skilled observer could not recognize them. It is obvious that the disease must be regarded as having begun at the moment when the bacillus, having gained entrance into the body, has set in motion that process, interruptable at any step to be sure, by which in the ordinary, "normal" course of events, recognizable degrees and forms of the disease will be produced. This reasoning may quite logically be applied to the case of carcinoma. It is apparent that the capacity for and tendency to invasive growth must be acquired before such

growth can take place; and since invasive growth is one of the characters that define the cancer cell, it is clear that the cancerous process is present before invasive growth begins, and from the moment when the series of changes leading to development of cancer is set in motion, beginning perhaps a long time before recognizable carcinoma tissue is present. Whether we can recognize a carcinoma or ever hope to recognize it at such a stage, has in the language of the old song "nothing to do with the case." That the ability to grow invasively does not of itself constitute carcinoma is clearly shown by such phenomena as the pseudocarcinoma or carcinoid condition of the appendix.

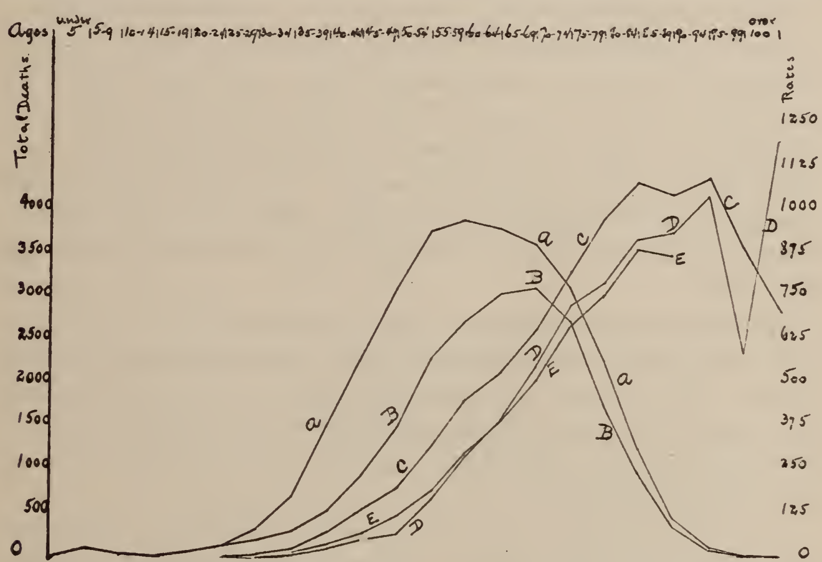
Third, the etiology of cancer becomes then the etiology of mutation. The law that appositional growth never takes place (Ribbert's *Aussichherauswachsen*), precludes the idea of a specific infectious agent, in the ordinary sense, for we must either assume that such an agent dies after having started the cancerous process but before invasion begins (a notion which, by the way, admits my contention that the cancerous disease is present before the cancer is there) or else that there exists a multitude of specific agents, one for each kind of tumor and for each kind of tissue; for example, one agent for carcinoma of the stomach and another for carcinoma of the liver. On the other hand, the development of cancer following the action of a considerable variety of nonspecific irritations (lupus, gastric ulcer, arsenic, paraffin, soot, tar, wind and weather, as in seaman's cancer) some of which (x-ray, radium) are known to have a specific action on the mitotic process, furnishes us with a much simpler and more comprehensible viewpoint. Whenever such an irritant excites over a long period, constantly renewed and constantly defeated efforts at repair, an enormously increased opportunity is obviously afforded for such an accident to occur during the mitotic process, as would produce the particular mutation required.

Ribbert, as is well known, has ascribed great importance to the degenerative and inflammatory changes usually present in the neighboring connective tissue. My cases show that these changes are not always present. Inflammatory infiltration of a certain degree may antedate *visible* changes in the epithelium,

but its intensity and extent increase up to a certain point, pari passu, with the further growth and spread of the tumor. The commonly observed absence of an inflammatory reaction in carcinoma of the fundus of the uterus can be adequately explained perhaps on the assumption that the location and character of the growth is such as to afford free drainage for the product of its own abnormal metabolism, so that no absorption of them takes place. That portion of the change which is not the result, rather than the cause, of the cancer may be quite reasonably explained as a pure coincidence, since it apparently extends over a vastly greater area than that involved in the tumor growth, and may be present where no tumor exists (cf. Unna, loc. cit.). Or we may regard these changes as part and parcel of the cancer process, as the result not the cause, of changes occurring in the epithelium, out of which the cancer eventually arises. At the most such changes might afford an increased opportunity for the sort of accident just mentioned, in so far as they antedate the cancerous process and in so far as their presence implies the presence of anomalies of metabolism.

I have already urged (loc. cit.) the possible importance of hybridization and mongrelization in the same direction. The same general considerations apply to the effect of old age. Attention has recently been drawn to the fact that there is no such thing as a "cancer age." For although it is true that the total number of deaths from cancer is greatest in the decades on either side of the (male as well as female) menopause, the cancer rate increases steadily throughout life and is greatest in those one hundred years old or over (text-fig. 2). In this figure the line *A* represents the total number of deaths of women from cancer by ages, in the United States registration area in 1914, as given in the Census Bureau's mortality tables for that year. Line *B* gives the deaths of men. Before age thirty and after age one hundred, the number of deaths for each sex, while not identical, is so nearly equal that the difference cannot be indicated in a graph of the scale I have used and no separation in these age limits is attempted. Line *C* is the death rate per 100,000 for women and line *D* the rate for men. Here the rate before age

twenty-five for women and age thirty for men is so low that it cannot be indicated with any accuracy, and is therefore omitted. The curves show a slight absolute drop in the rate for women in the age group from eighty-five to eighty-nine, and a relative drop for men in the same age group. There is also a decided fall in the death rate for women after age ninety-five, and a sharp drop in the rate for men between ninety-five and ninety-nine, with a subsequent sharp rise in the group over one hundred to the highest point in the curve. These curves were obtained



TEXT FIG. 2

Curve A = total deaths in women, by ages, from cancer in 1914.
Curve B = total deaths in men, by ages, from cancer in 1914.
Curve C = death rate per 100,000 in women, from cancer in 1914.
Curve D = death rate per 100,000 in men, from cancer in 1914.
Curve E = death rate per 100,000, both sexes, from cancer in 1914. Figures furnished by Bureau of the Census.

by dividing the total number of deaths as given for 1914, by the total number living in each group, in the same area, as given for 1910. They are therefore "crude" rates, but could not be corrected since the Census Bureau was unable to furnish corrected populations for that year. I can explain the drops in the rates

above mentioned only on the assumption that they are due either to an unexpected change in the total number living in the age groups involved or, more probably, to the fact that the total number living in these age groups and the number of deaths were so small as to produce wholly misleading results. Rates determined in the same way for the year 1913 give a like result. Line *E* was obtained by reducing to graph form a table giving the joint rate for both sexes, published by the Census Bureau after I had prepared the rest of the figure. That table extends only from age thirty-five to age ninety. Between the ages of thirty-five and sixty-five it lies, as would be expected, between the lines for the sexes as I plotted them. After age sixty-five it lies below both my curves. This result is perhaps due to the fact that my rates are, as already mentioned, crude rates, while the Bureau's are doubtless corrected. The difference is wholly unimportant in the present discussion. It will be noted that the Bureau's table also shows a slight drop in the rate after age eighty-five.

The fact that the cancer rate rises throughout life is in harmony with the idea that the likelihood of a disturbance in the enormously delicate and complicated process of mitosis increases the longer we live, in accordance with the law of chance. Possibly the increasing sluggishness of function incidental to old age adds a favoring factor.

The same applies to the parakeratotic process to which also Ribbert attaches importance. It is at least as rational to assume that these are an expression of a mutative change in the life of the cells, representing a step in the process to which the cancer owes its origin.

SUMMARY

1. The carcinoma cell is a new variety (or better, species) of cell, arising by somatic mutation from a normal cell existing either as a cell rest, or normally intercalated in the tissues of the host, and retaining in almost every case enough of the properties of the parent cell to render its point of origin recognizable within certain limits.

2. The cause of such mutative changes can only be surmised; but apparently they are favored by hybridization and mongrelization, and are more immediately brought about by chronic irritations which maintain long continued efforts at repair—particularly when such irritants exercise, as in the case of *x*-ray and radium, a specific power to interfere with the mitotic process.

3. Such mutations are apt to recur from time to time in the growing tumor, bringing about a progressive loss of the original character of the mother cell, and change in the clinical behavior of the tumor.

4. It follows from 3, that in many cases the biologic properties of a carcinoma are not constant or uniform, but vary from time to time and region to region of the tumor.

5. It further follows from 3 and 4, that since the histogenesis of a carcinoma is a gradual process, the real beginning of this process must actually antedate visible changes in the cell, by perhaps a long period.

6. The particular characteristics of squamous-cell carcinomata do not depend upon the tissue of origin, but upon the character of the responsible mutative change.

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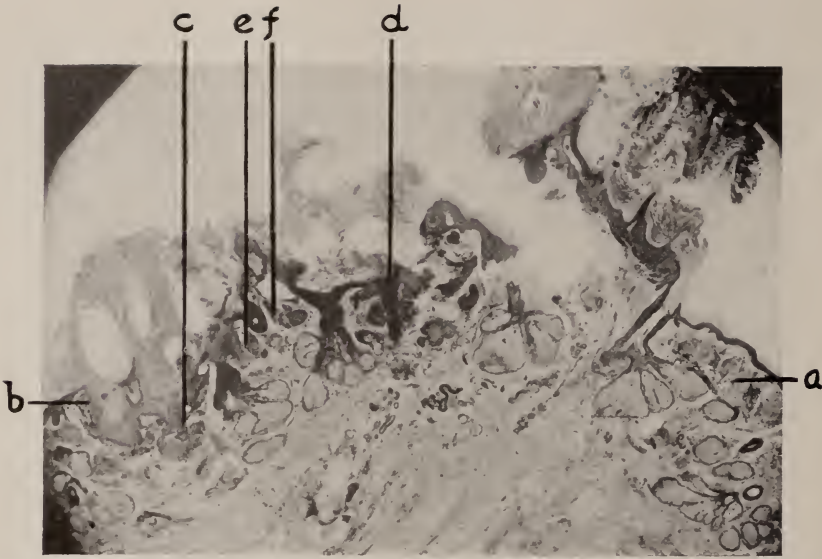

Fig. 1

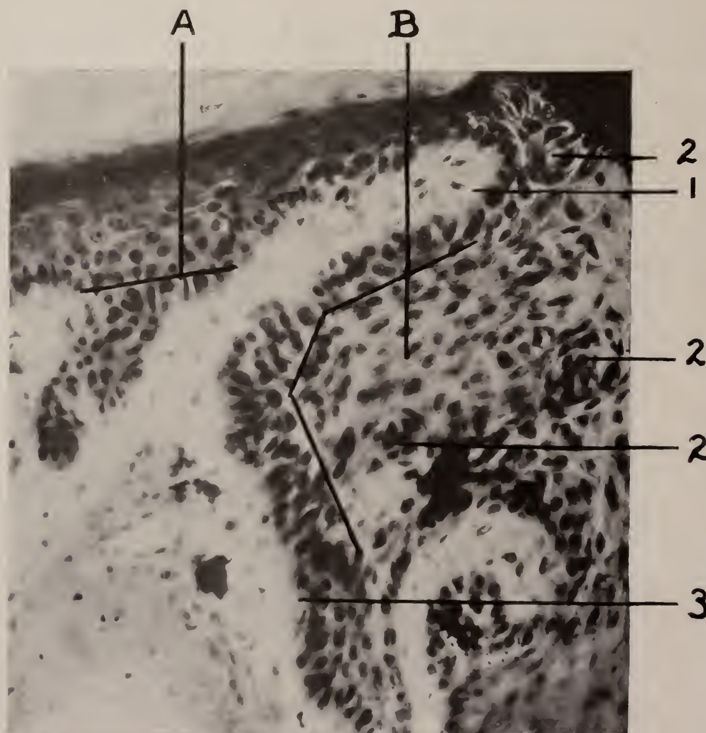

Fig. 2

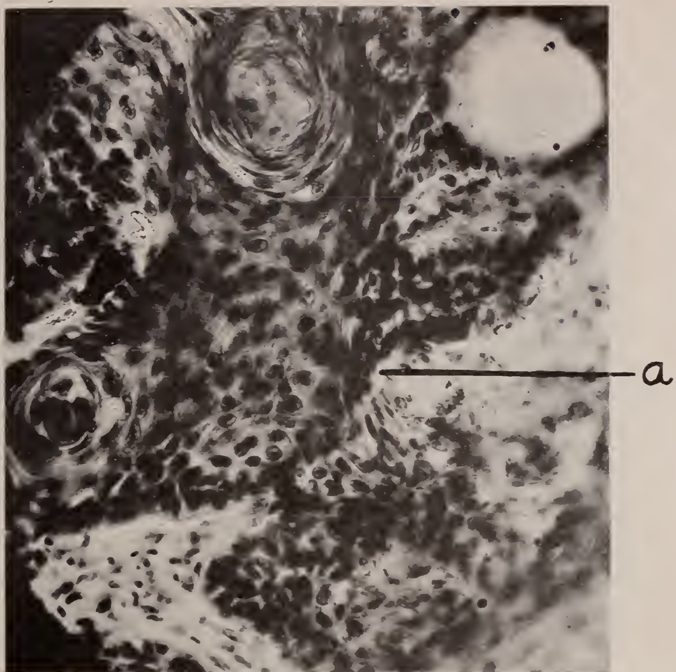

FIG. 3

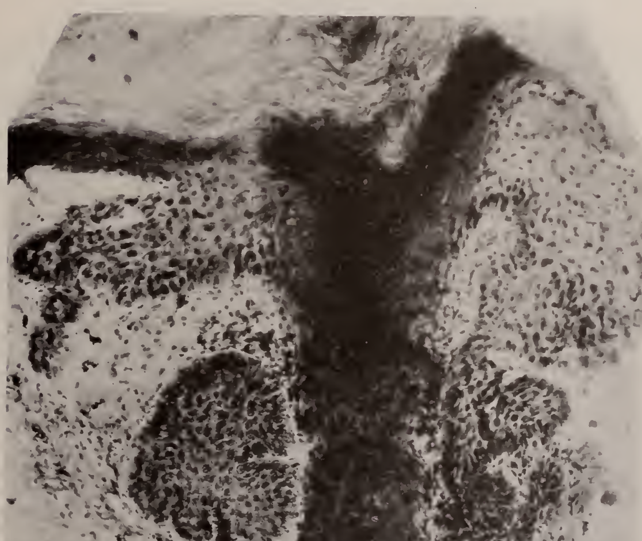

FIG. 4

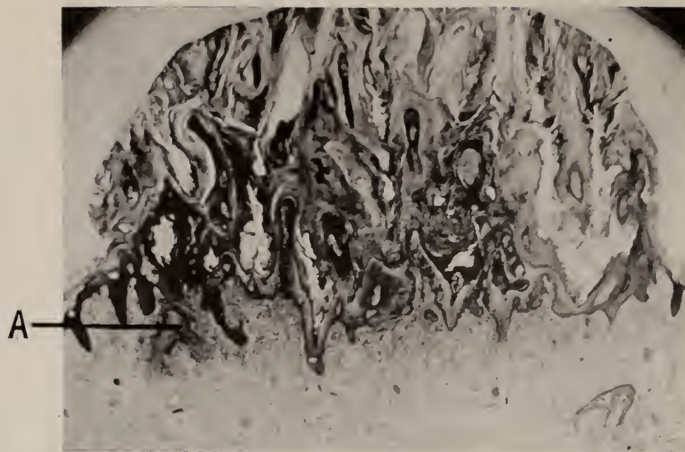

Fig. 5

Fig. 6

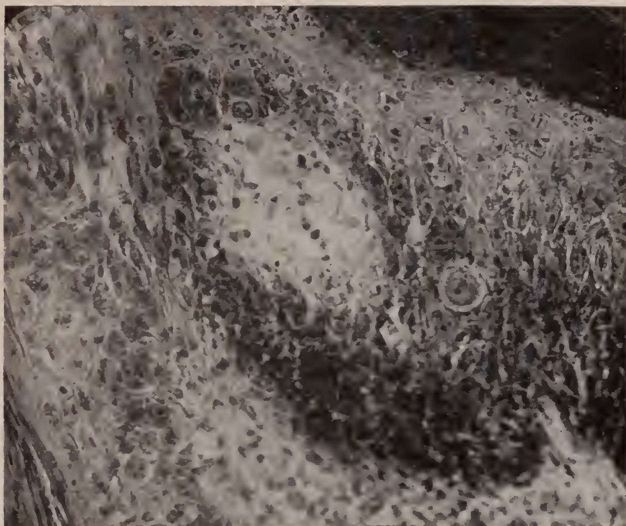

FIG. 7

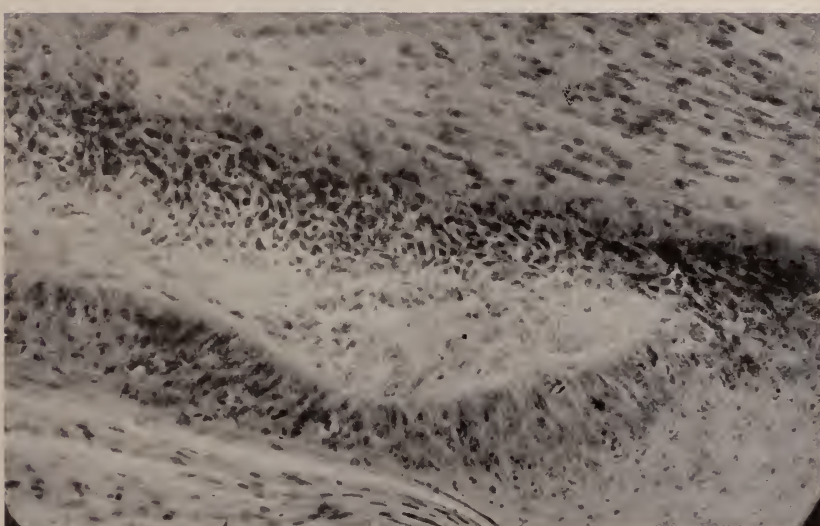

FIG. 8

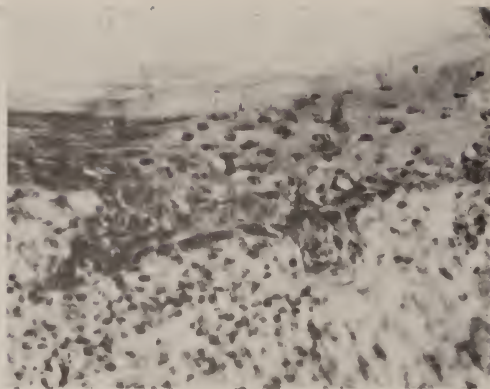

Fig. 9

Fig. 10

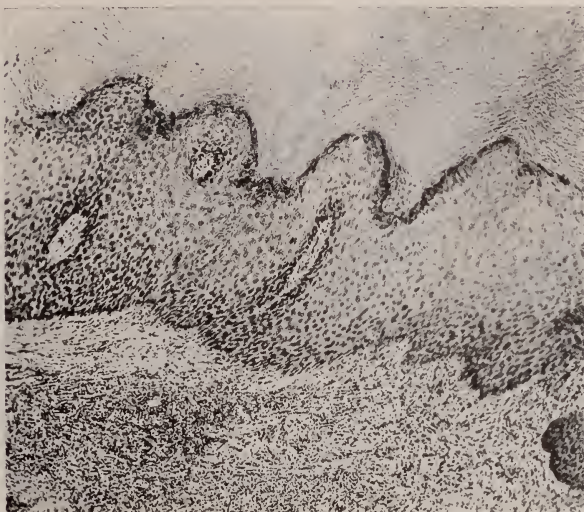

FIG. 11

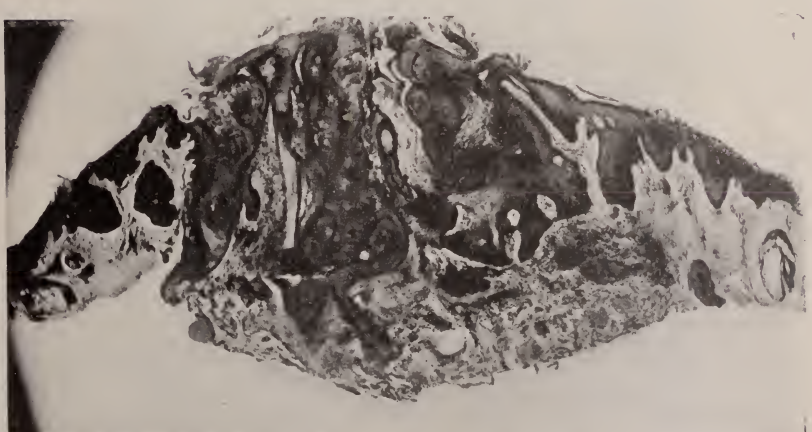

FIG. 12

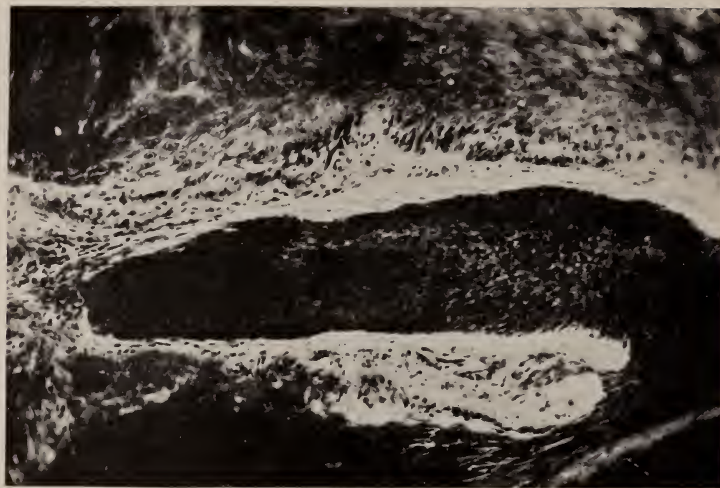

Fig. 13

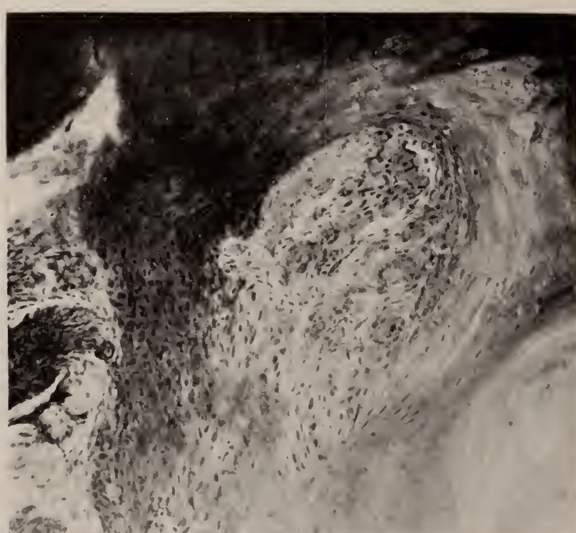

Fig. 14

FIG. 15

FIG. 16

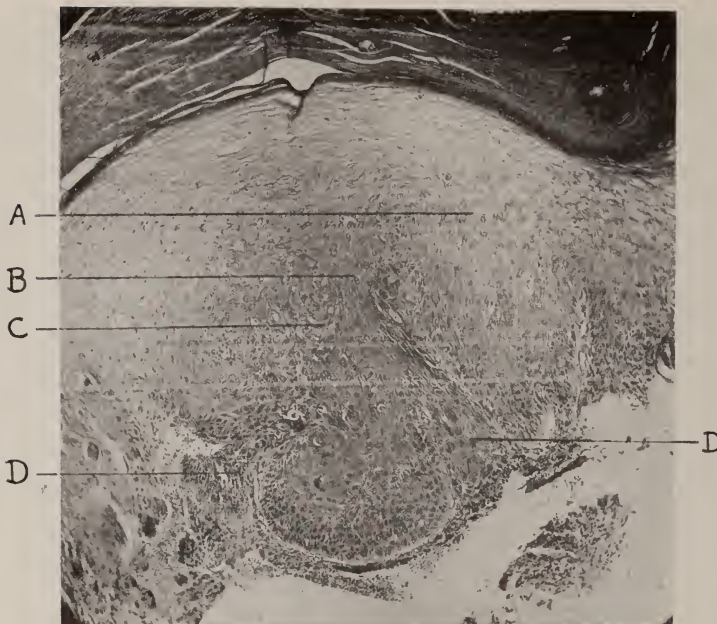

Fig. 17

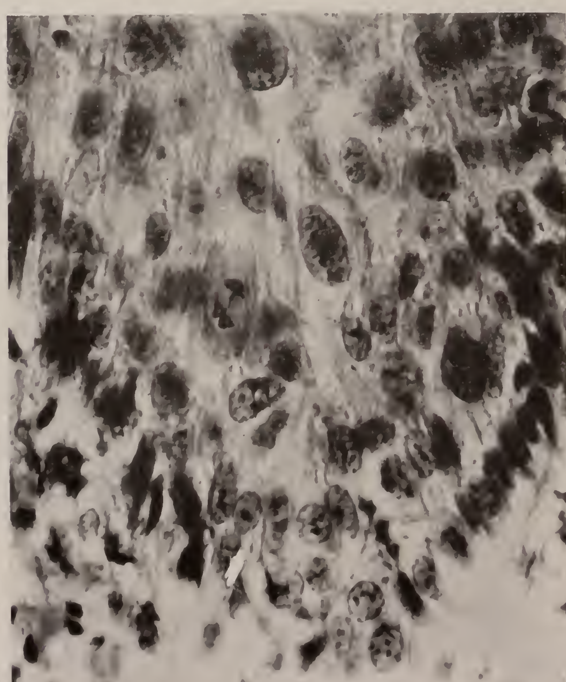

Fig. 18

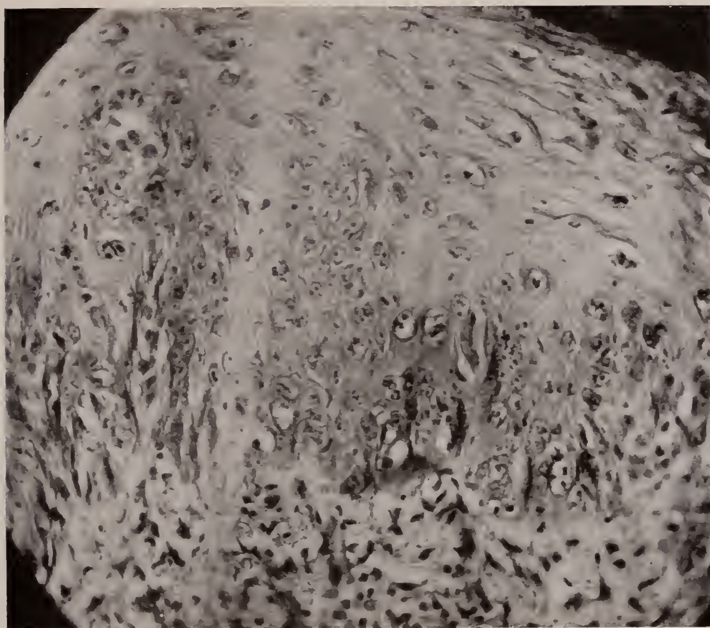

Fig. 19

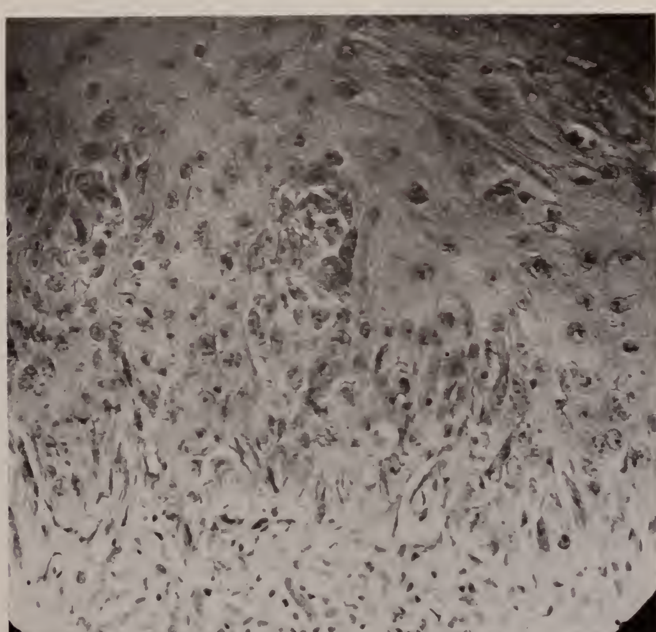

Fig. 20

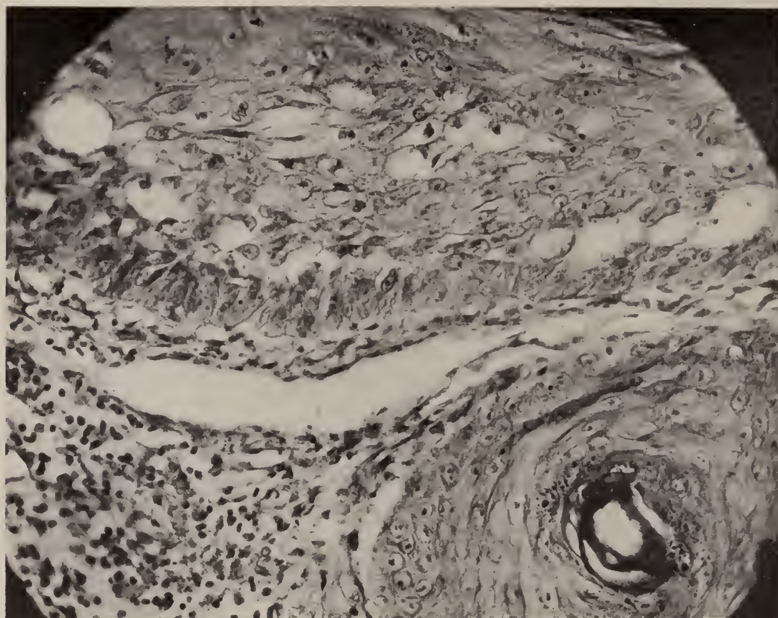

Fig. 21

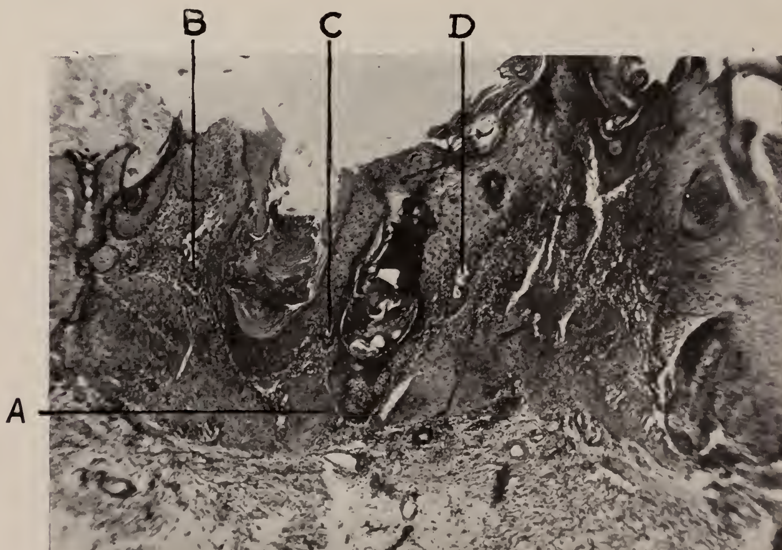

Fig. 22

Fig 23

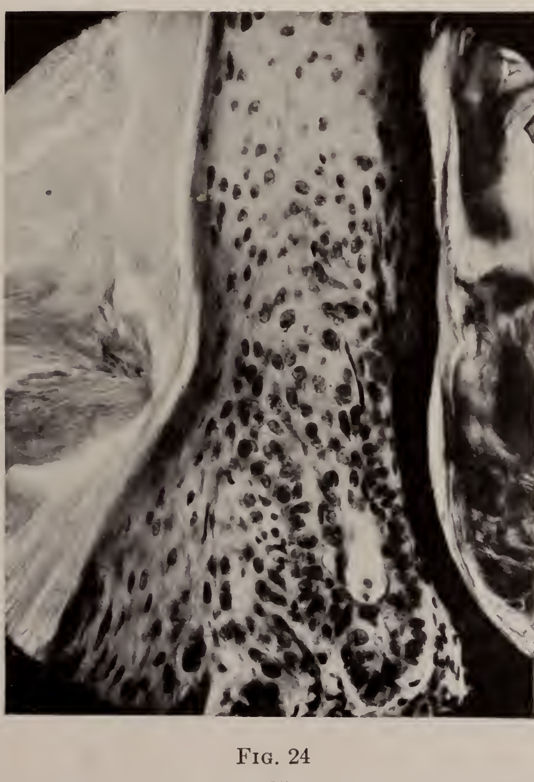

Fig. 24

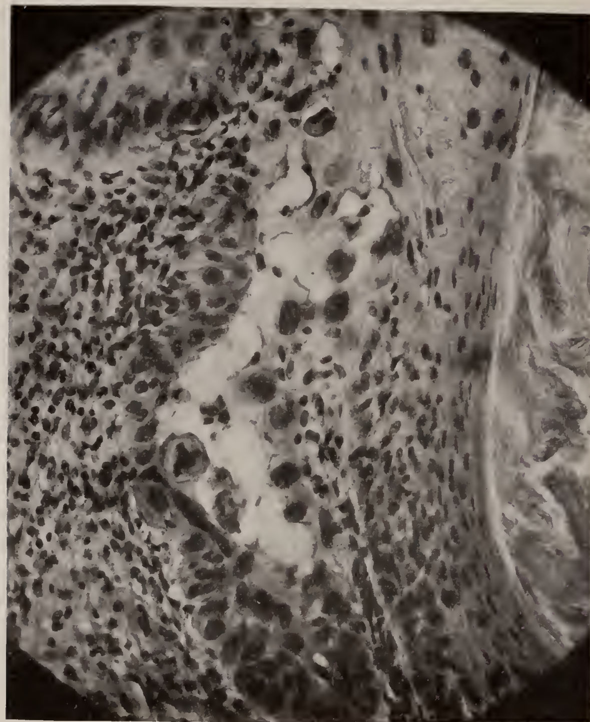

Fig. 25

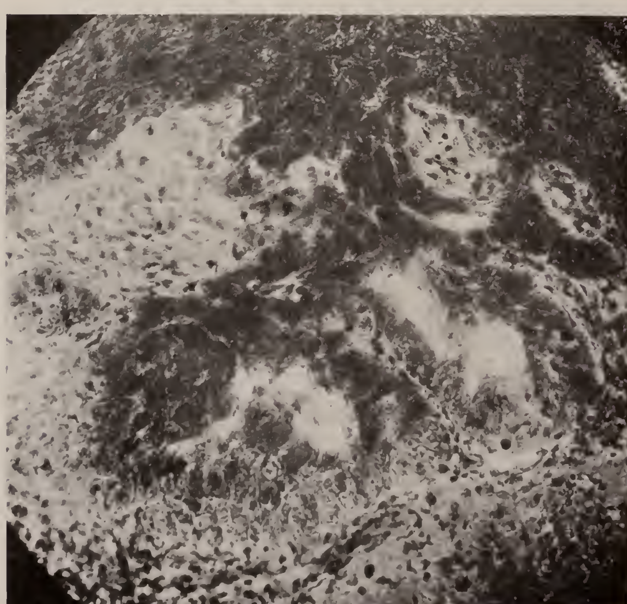

Fig. 26

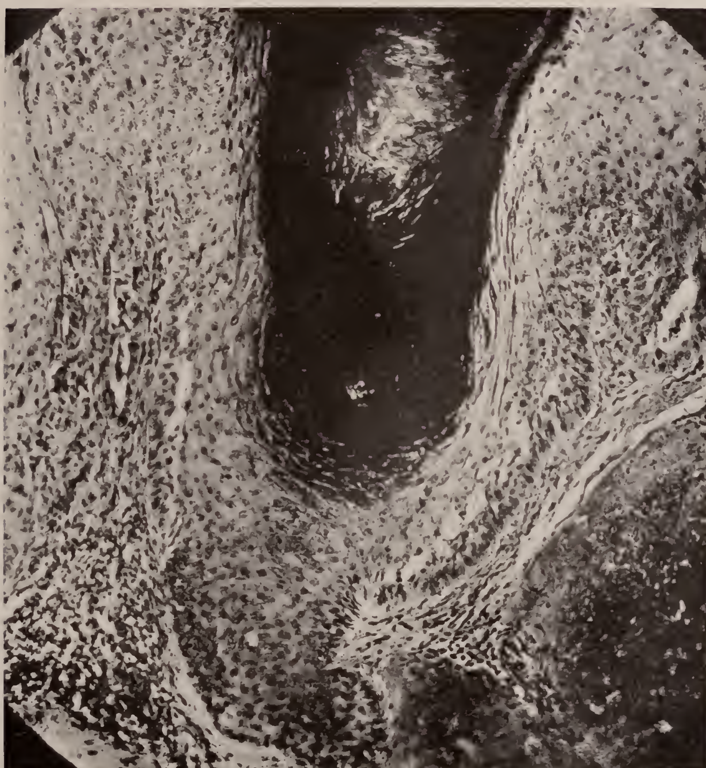

Fig. 27

ON THE CALCIUM CONTENT OF THE BLOOD WITH SPECIAL REFERENCE TO CANCER

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In his studies on the chemistry of malignant growths, Beebe (1) found that old degenerated carcinomata and sarcomata contained much more calcium than did young, rapidly growing tumors. Likewise, Clowes and Frisbie (2) observed a low calcium and high potassium content in rapidly growing mouse tumors, and the reverse in tumors of slow growth. These observations suggested the possibility of a disturbance of calcium metabolism in neoplastic disease; and, with a view to demonstrating such a disturbance, the determination of the blood calcium was undertaken in a series of cancer cases.

As a basis for comparison with the values obtained in cancer, the calcium content of the blood of a number of patients presenting no evidence of malignant disease, was ascertained.

The method employed was that of Halverson and Bergeim (3). The technique of these authors was closely followed, except that the protein precipitation was done in a 50 cc. centrifuge tube and centrifugalization resorted to in order to facilitate removal of the protein precipitate. Duplicates were done in all cases.

The data of thirty-four patients with malignant disease are presented in table 1. The calcium content of the blood plasma varied from 5.87 to 15.05 mgm. per 100 cc., the average being 9.41 mgm. If the type and location of the tumor are disregarded, the calcium concentration of the plasma of the male is slightly lower than that of the female, the mean figure for the former being 9.42 mgm., for the latter 10.21 mgm. These average values may be considered to be within normal limits, Howland and Marriott (4) having found from 10 to 11 mgm. per 100 cc., and

Halverson, Mohler, and Bergeim (5) from 9 to 11 mgm. in a series of normal individuals. In 64 per cent of the inoperable abdominal cases, the calcium content exceeded 10 mgm., but a truly characteristic calcium value for any definite type or location of

TABLE 1

	SEX	DIAGNOSIS	CALCIUM PER 100 CC.
			mgm.
1	M	Epithelioma of face	8.28
2	M	Epithelioma of face	5.87
3	M	Epithelioma of back	10.96
4	F	Carcinoma of breast	8.04
5	F	Carcinoma of breast	8.20
6	F	Carcinoma of breast	9.35
7	F	Carcinoma of breast	8.50
8	M	Abdominal carcinoma*	11.60
9	M	Abdominal carcinoma	10.41
10	M	Abdominal carcinoma	15.05
11	F	Abdominal carcinoma	14.24
12	M	Abdominal carcinoma	10.30
13	F	Abdominal carcinoma	11.67
14	M	Abdominal carcinoma	10.41
15	F	Abdominal carcinoma	13.40
16	F	Abdominal carcinoma	10.79
17	M	Abdominal carcinoma	10.71
18	M	Abdominal carcinoma	9.08
19	M	Abdominal carcinoma	9.11
20	M	Abdominal carcinoma	6.02
21	M	Abdominal carcinoma	6.18
22	M	Abdominal carcinoma	7.10
23	M	Abdominal carcinoma	9.61
24	M	Abdominal carcinoma	10.51
25	M	Carcinoma of stomach	11.59
26	F	Carcinoma of stomach	9.04
27	M	Carcinoma of esophagus	7.61
28	M	Carcinoma of rectum	8.28
29	M	Carcinoma of bladder	6.44
30	M	Carcinoma of bladder	10.89
31	F	Carcinoma of uterus	10.40
32	M	Carcinoma of vocal cord	10.04
33	M	Sarcoma of thymus	10.74
34	F	Sarcoma of uterus	9.68

* Abdominal carcinoma indicates generalized peritoneal metastases, primary tumor not located. All diagnoses confirmed by histological examination.

TABLE 2

	SEX	DIAGNOSIS	CALCIUM PER 100 CC.
			mgm.
1	M	Thromboangiitis obliterans	10.51
2	M	Thromboangiitis obliterans	5.41
3	M	Thromboangiitis obliterans	9.87
4	M	Thromboangiitis obliterans	8.06
5	M	Thromboangiitis obliterans	10.80
6	M	Thromboangiitis obliterans	11.51
7	M	Thromboangiitis obliterans	12.34
8	M	Thromboangiitis obliterans	9.90
9	M	Thromboangiitis obliterans	8.65
10	M	Thromboangiitis obliterans	11.97
11	M	Thromboangiitis obliterans	12.21
12	M	Multiple exostoses	10.58
13	F	Cystic goiter	12.51
14	M	Congenital cystic kidney	7.65
15	F	Cystadenoma of ovary	10.29
16	F	Cystadenoma of ovary	8.65
17	F	Papilloma of bladder	11.21
18	F	Pulmonary tuberculosis	14.31
19	M	Healed pulmonary tuberculosis	8.73
20	M	Cholecystitis	9.54
21	M	Cholecystitis	11.12
22	M	Pneumonia (ante mortem)	8.23
23	M	Abscess of lung	11.43
24	F	Chronic appendicitis	8.20
25	F	Syphilis	10.54
26	M	Chronic nephritis	9.51
27	M	Chronic nephritis	7.80
28	M	Chronic nephritis	11.40
29	F	Eclampsia	4.29
30	M	Acromegaly	7.70
31	F	Acromegaly	13.33
32	F	Exophthalmic goiter	10.66
33	M	Diabetes	12.68
34	M	Diabetes	9.58
35	M	Pernicious anemia?	8.69
36	F	Cerebral degeneration, arteriosclerosis	9.22
37	M	Gastroptosis	11.15
38	F	Neurosis	11.38
39	M	Neurosis	8.26
40	F	Bronchial asthma	8.96
41	F	Obscure abdominal condition	10.51
42	M	Tumor of lung?	9.89
43	M	Tetany	6.11

neoplasm is not in evidence. In six benign tumors (table 2) the results were similar to those obtained in malignant disease; they varied from 7.65 to 12.51 mgm., and averaged 10.14 mgm.

Table 2 also records the calcium figures of eleven cases of thromboangiitis obliterans, as well as the clinical diagnoses and blood calcium values of twenty-six miscellaneous cases, in which no sign of malignant disease was manifest. In the miscellaneous material, no one disease is represented by a number of cases sufficiently large to permit of definite conclusions regarding changes in the calcium concentration that may occur; the figures are presented, however, because additions to the blood calcium data available at present, seem indicated.

For thromboangiitis obliterans, the average calcium concentration in the blood is again within normal limits (10.11 mgm. per 100 cc.), while the variations in individual cases (5.41 to 12.34 mgm.) would tend to show that a relationship between this disease and calcium metabolism does not exist.

Regarding individual cases, it may be of interest to note that only 4.29 mgm. of calcium were found in a case of eclampsia, and that 6.11 mgm. occurred in a typical case of tetany, the latter figure in accord with those observed by Howland and Marriott (6). In chronic nephritis our results agree with those of Halverson, Mohler, and Bergeim (7), a low figure of 7.8 mgm. occurring in a case with retention, while two of a milder type presented concentrations within normal limits. On the other hand, 14.31 mgm. occurring in one of our tuberculous patients, is rather high as compared to the figures obtained by Halverson, Mohler, and Bergeim in their study of thirty cases of tuberculosis.

SUMMARY AND CONCLUSIONS

The blood plasma calcium content was determined in thirty-four cases of malignant disease, in six of benign tumors, in eleven of thromboangiitis obliterans, and in twenty-six miscellaneous cases.

In cancer the average calcium values were within the figures generally accepted as normal, and no characteristic concentra-

tion accompanied any given type or location of neoplasm. In benign tumors the results were similar.

The average calcium figure for thromboangiitis was within normal limits, while the variations in individual cases would indicate that calcium metabolism has no connection with this disease.

In accordance with the results of other authors, low calcium values were obtained in severe nephritis, in eclampsia, and in tetany.

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PRIMARY SPONTANEOUS TUMORS OF THE OVARY IN MICE

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Fourteenth Communication

MAUD SLYE, HARRIET F. HOLMES AND H. GIDEON WELLS

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Throughout the animal kingdom the solid ovarian tumors seem to be infrequent, as they also are, relatively, among human tumors. Cystic tumors are described occasionally, but apparently less frequently in the lower animals than in man. Even among dogs, with their high incidence of tumor growth, ovarian tumors are rare according to the evidence furnished by literature dealing with canine neoplasms.[1] In Sticker's (2) compilation of tumors in domestic animals, of 766 tumors in dogs but 3 were in the ovary. As to other animals of Sticker's series, in 509 cases of tumors in horses, 4 were in the ovary; of 110 in cattle, 6 were ovarian; there was 1 ovarian tumor among 23 tumors in cats, and none at all among sheep, goats, and swine. Kimura (3) has reported 142 cases of tumors in horses, among which were no ovarian tumors, although there were 49 in the testicle. Other evidence supports the figures of Sticker in indicating that cows have ovarian tumors more often than other species. Trotter (4) reported 305 bovine tumors observed in the Glasgow slaughter house, of which 5 were in the ovary (4 carcinomas and

[1] Goodpasture (1) notes the occurrence of small hyperplastic areas in the senile ovaries of old dogs, but even these do not seem to be of very frequent occurrence in proportion to the high incidence of proliferative changes noted by him in other tissues.

1 sarcoma). These were all large lobulated solid tumors, and no metastases were observed in any. Leo Loeb (5) has described a tumor arising in the ovary of a six-months-old calf, composed chiefly of cells resembling luteum tissue.

Among the numerous instances of tumors in wild rats reported by McCoy (6), Woolley and Wherry (7), and Beatti (8) there is no case of ovarian growth; nor have we found reports of any cases occurring in domesticated rats.[2]

Wolff (9) mentions 2 cases of ovarian tumors in cats: One a primary carcinoma in the ovary of a thirteen-year old cat with metastasis in the liver, reported by Kitt; the other a sarcoma of the ovary and pelvis reported by Stroud.

Wild animals are also unlikely to have ovarian tumors. Fox (10), in his extensive studies of tumors in wild animals, has described no cases whatever of ovarian tumors among the mammalia.

Only in birds do ovarian tumors seem to be relatively frequent. Bürger (11) found, among 852 fowls autopsied at the Leipzig veterinary institute, 12 tumors, of which 7 were in the ovary, 4 being sarcomas and 3 carcinomas; 2 of the sarcomas and 2 of the carcinomas had produced metastases. In their review of the literature on tumors in birds, Joest and Ernesti (12) found 112 cases reported and added 50 more. Of these 162 cases, 21 were primary carcinoma of the ovary, commonly with extensive peritoneal and visceral metastasis. There was also one case of ovarian sarcoma.

An ovarian tumor has been described in a wild turkey (*Meleagris gallapavo*) by Fox (10), as follows: The growth was "about the size of 3 English walnuts placed in triangular position." It consisted of 3 subdivisions, covered with papillomatous prominences like a hydatid mole. Microscopically it was a papillary cystadenoma.

[2] While this article was in press there appeared under the title "Carcinosarcoma de rata blanca," an article by Dr. A. H. Roffo (Revista del Instituto Bacteriologico, Buenos Aires, 1919, ii, 283), reporting the finding of a large tumor in the ovary of a white rat. This seems, from the illustration and description, to have been a papillary cystadenoma with areas of more compact cell growth, some of which are interpreted as carcinoma and some as sarcoma.

Cold-blooded animals also have furnished occasional cases of ovarian tumor. Bland-Sutton (13) has reported a case of tumor of the ovaries of a python, with growths also in the lungs, liver, and peritoneum; he believed the ovarian growth to have been primary but without conclusive evidence. Plehn (14) has described tumors arising in the ovary of an old grass frog (*Rana esculenta*) through growth of primitive ova cells without differentiation. There were numerous nodules, from millet seed to cherry size, apparently benign in character although resembling a malignant tumor in histological structure. A cystic tumor of the ovary has been described in a fish, the ling (*Molva molva*) by Johnstone (15).

In mice, also, ovarian tumors are not common, but we have found mention of 8 cases in the literature. The first 2 cases were described by Jobling (16). One was bilateral, each ovary being eight times the normal size. Both ovaries showed the same structure, which is described as follows:

> A great increase in epithelial cells, which formed solid masses, somewhat compressed and elongated into spindle cells and cysts of different sizes, usually small and more or less occupied by the papillary outgrowths from their walls. These outgrowths developed from narrow or somewhat thickened pedicles and spread out into a fanlike structure. . . . . The more solid portions were at one time cystic but the cysts became occluded by the ingrowth of papillae. Acini possessing a distinctly granular [sic., glandular?] form and arrangement also occurred. Mitosis was rare, direct division more frequent. Hemorrhage had taken place into some of the cysts. Mallory stain showed a delicate connective-tissue basement membrane surrounding the small cysts and the more solid areas, the latter being filled with epithelial cells of a granular quality resembling somewhat the lutein cells.

In the other case the growth was unilateral, there being numerous large cysts, separated by smaller ones and by the tubules of the ovary, lined by high columnar epithelium without cilia. Between the cysts the tissue was composed largely of smooth muscle fibers, resembling in places a leiomyoma.

Tyzzer (17) has reported 4 cases. 1. This tumor was bilateral, the ovaries being replaced by an irregularly glandular epithelial growth, in places with flattened epithelial cells intimately mingled with the connective tissue; in others the glands contained thick papillary processes of epithelium without central connective tissue core protruding into the lumen, the epithelium being in places high columnar, in others merely a fused mass. There were no mitotic figures, but the presence of masses of epithelium in the lymphatics suggested malignancy. 2. In a mouse with a renal tumor resembling a hypernephroma, one ovary was replaced by an irregular gland-like structure similar to the above. 3. A mouse of a series bred for heredity studies, which died from a lung tumor, had the right ovary replaced by a mass the size of a large pea, composed of irregular epithelium having in places a partially glandular structure, without tendency to form spaces. 4. A mouse from the same family as (3), had both ovaries replaced by masses of translucent whitish tissue, 5 by 3 by 3 mm. This also had the structure of a papillary adenoma. Tyzzer notes that these growths differ from the ordinary types of ovarian tumors seen in human beings, in that the epithelium is more or less glandular with but little tendency to form cysts. The epithelium resembles the peritoneal mesothelium found at the attachment of the ovary.

Haaland (18) found 2 ovarian tumors among 353 primary tumors observed in 288 mice, of which 325 were mammary gland growths. 1. A mouse that had a sarcoma arising in a scar also had a tumor the size of a hazel nut in the left ovary. The ovarian tumor was composed of large alveoli with a peripheral layer of low columnar epithelial cells, the lumen filled with round cells and sometimes exhibiting spaces filled with serous fluid. This tissue was transplanted into 120 normal mice, but no growths resulted. 2. A mouse that had been operated for mammary carcinoma with recurrence. A tumor "as large as a pea" replaced the right ovary, and in structure was a papillary adenocarcinoma, probably primary.

Of these 8 recorded cases of ovarian tumors in mice there were 3 cases of bilateral tumors, although in no case was there metas-

Fig. 1. Solid Tubular Adenoma

This is the most usual type of benign tumor of the ovary in mice. Much of what seems to be stroma between the tubules is really composed of compressed epithelial cells. Mouse 12760. × 110.

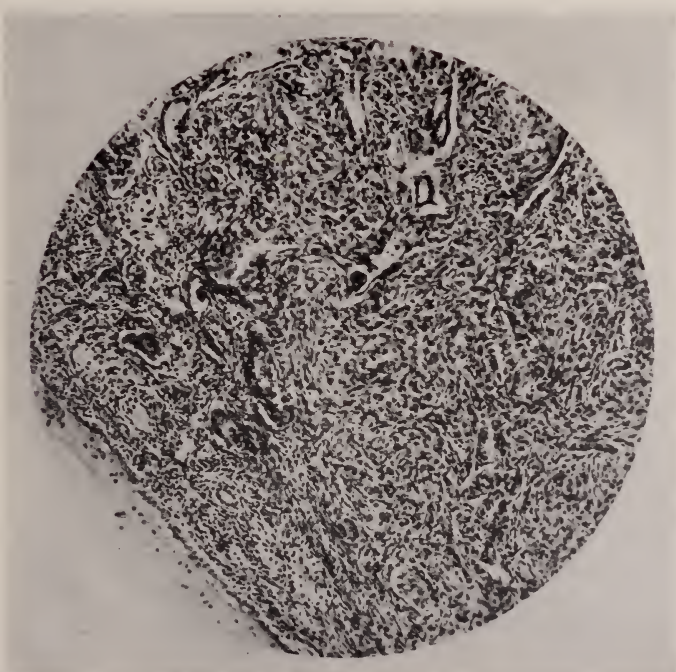

Fig. 2. Solid Tubular Adenoma

Showing a larger proportion of compressed epithelial masses. × 110

tasis or other posirive evidence of malignancy. Four of the eight mice had tumots elsewhere than in the ovaries. All but one of the tumors were of similar structure, consisting of atypical glandular or alveolar formations with a tendency to epithelial proliferation into the lumen to form solid plugs or papillae—apparently best described as solid, atypical papillary adenoma.

Among 22,000 mice of the Slye stock that have come to autopsy have been found 46 with solid tumors that seemed to be primary in the ovary. This may be compared with 28 testicle tumors in 19,000 autopsies, 160 lung tumors in 6,000 autopsies, 87 sarcomas in 12,000 autopsies, and 4 cancers of the stomach in 16,500 autopsies.[3] These figures indicate that, as with other animals, solid ovarian tumors are not common in mice. There have been observed simple cystic conditions in the ovaries of but a small number of mice, and ovarian cysts are apparently rather infrequent. The strikingly large cysts seen in women have never been observed. In only a few of the

[3] We would again emphasize the character of the material from which these tumors have been obtained, and the conditions under which the growths have developed. The 22,000 mice are all the descendants of a limited and carefully selected stock, bred together according to definite plans designed to give evidence as to the influence of heredity upon the incidence of spontaneous tumors in mice, and, hence, including strains of highly cancerous ancestry and strains with ancestry free from cancer. They represent strains in which cancer is very common, strains in which it never occurs, and strains of intermediate character. The influence of heredity on the incidence of ovarian tumors will be considered elsewhere, and we mention these facts here to indicate the character of the material in this respect. It must also be emphasized that none of these mice has been subjected to any artificial influences that might modify its life. In no case is a spontaneous tumor used for inoculation, or operated upon, and no mouse born in this laboratory is ever used for any experimental work whatever. From the moment of its birth every effort is directed to the one object of permitting each mouse to reach a maximum age. Long experience and great care have made it possible to limit to a large extent the epidemic infections that constantly threaten such large colonies of mice under even the best of conditions. Of especial importance is the fact that every mouse that dies is submitted to a careful post-mortem examination, no matter whether it dies in infancy, from an accident, or from any other obvious cause; and every suspicious area is submitted to microscopic examination by three people or more. Were it not that every dead mouse is thus thoroughly investigated, and that the average age at death is, for a mouse community, very high, we should not have nearly so much material to describe.

Fig. 3. Solid Tubular Adenoma

In this growth there is an unusually large proportion of ovarian stroma type. × 76.

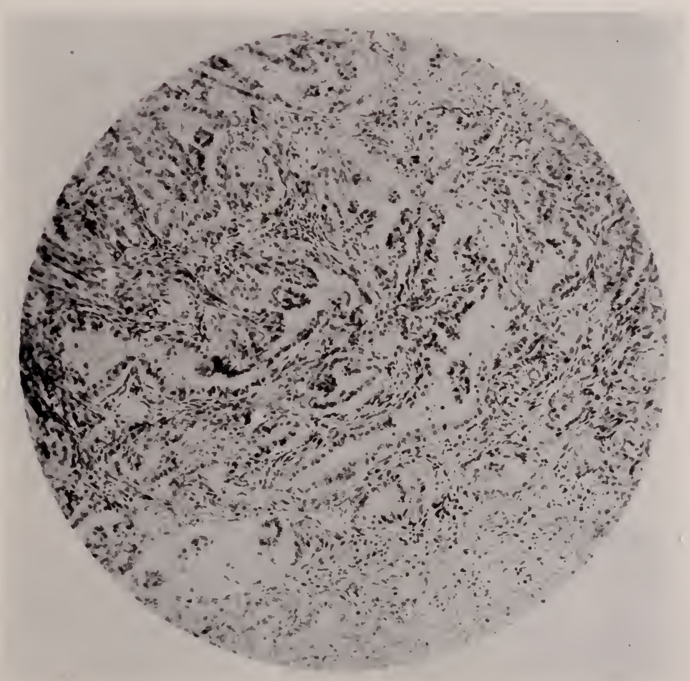

Fig. 4. Papillary Portion of a Growth that Elsewhere is a Solid Tubular Adenoma, as in 1 and 2

Small areas thus disclosing an existing tendency to papillary structure are not infrequently found in the ordinary solid ovarian tumors of mice. Mouse 2205. × 110.

ovarian cysts that we have examined have small cystic papillomatous areas been found. Therefore, mouse ovarian tumors present a marked preponderance of solid adenomatous growth, as compared with the proportion of cystic ovarian enlargements in women. We have eliminated from our consideration the simple cysts, as probably not examples of neoplastic growth, with the exception of those cysts that result from secretion by a cystadenoma.

Most of our tumors seem to be benign in character, although we have found a few examples of undoubted malignant tumors primary in the ovary. In all, 38 mice have exhibited tumors that may be classified as solid benign ovarian tumors, and there was one typical papillomatous cystoma. As 19 of these had bilateral tumors there are 58 ovarian tumors occurring in a stock of mice that have yielded over three thousand primary spontaneous tumors of other tissues, chiefly the mammary gland.

BENIGN OVARIAN TUMORS

In structure these tumors vary considerably, although most of them correspond closely to the descriptions given by Jobling, Tyzzer, and Haaland, and may be most appropriately designated as solid alveolar adenoma. Tendency to cyst formation is exceptional, in contrast to the adenomas of the human ovary, and the same is true of papillary types of growth, which are rarely exhibited. In general these solid adenomas of the mouse ovary exhibit a growth apparently under great pressure, with crowding of the cells so that it is usually difficult to differentiate readily between stroma cells and flattened epithelial cells (see figs. 1 and 2). Probably this crowding of the growth accounts for the absence of papillary tendency, for often a small area may be found where there is less pressure or where part of the tissue has been destroyed by hemorrhage or necrosis, in which a distinct tendency to papillary structure is seen (see fig. 4). We have only one case (12111) in which the structure corresponds at all closely with the human papillary cystoma (fig. 7).

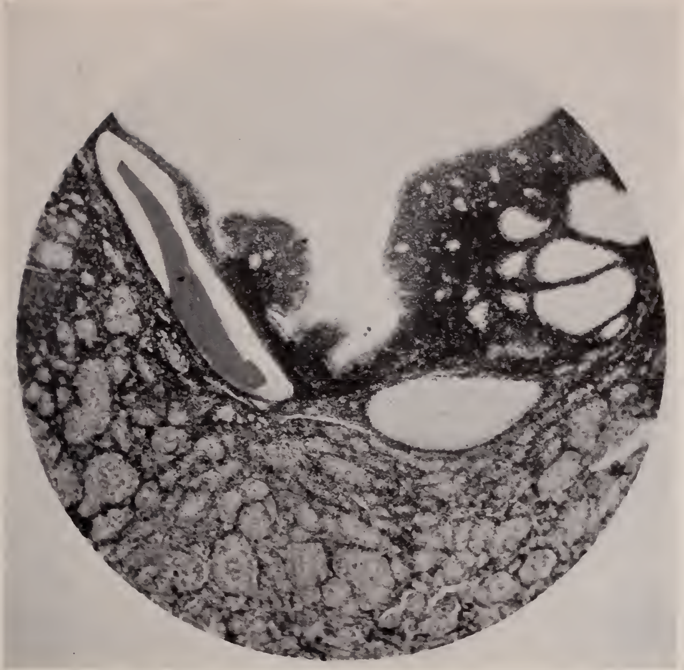

Fig. 5. Ovarian Tumor Exhibiting Cystadenoma Type of Growth in One Portion, While Another Part Shows a Solid Alveolar Character Resembling Primitive Follicles

Mouse 6991. × 50

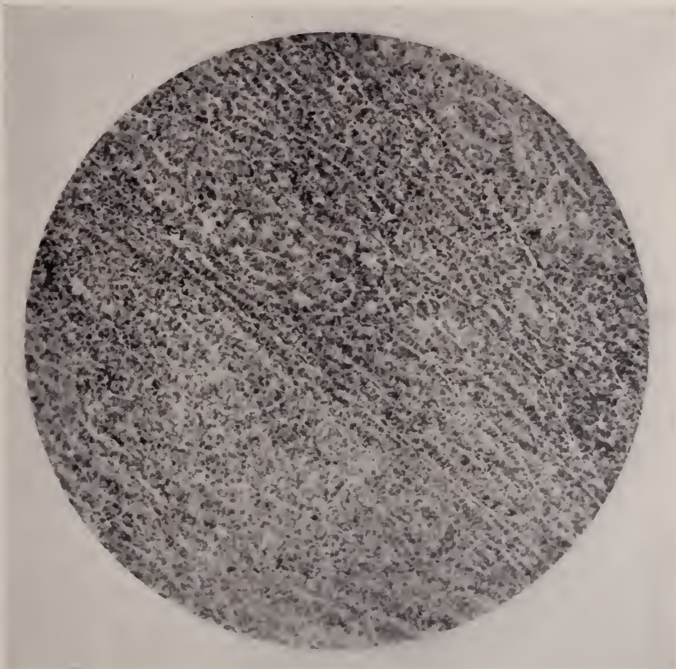

Fig. 6. Solid Tubular Adenoma

Consists throughout of a solid compressed mass of epithelial tubules, with a minimum of stroma. Mouse 20207. × 110.

Except for the few malignant growths in this series, nearly all the tumors seem to be fundamentally similar growths, yet differing considerably from one another according to the degree of differentiation permitted by the pressure under which the cells are growing. The usual character may be described as follows: The affected ovary is as a rule, uniformly enlarged, commonly to a diameter of 3 to 10 mm., white, firm, and often with a lobulated surface. In half of the cases the growth is bilateral, (19 of 38), usually one of the ovaries being considerably larger than the other.[4] The capsule is distinct but there may be adhesions to the adjacent tissues. If cysts are present they usually contain a clear watery fluid, but may have a blood-stained content. In most cases the benign ovarian adenomas have caused no apparent trouble; usually they are autopsy findings in mice dying from some unrelated condition. Sometimes fatal hemorrhage has occurred from ovarian tumors. In passing, it may be mentioned that large hematomas often form in the ovaries of mice without neoplasms, the blood usually forming strikingly laminated clots.

Microscopically the enlargement is usually caused by a compact growth of cells of two types, low cuboidal epithelial cells and spindle-shaped cells resembling those of normal ovarian stroma, but which often are definitely, in part at least, composed of compressed epithelial cells (see figs. 1 and 2). The cuboidal cells may form tubules, or solid plugs resembling the "Pflüger's tubes," or solid alveolar masses which may resemble primitive follicles, (see fig. 5), or occasionally alveoli into which papillary or fan-shaped outgrowths of the lining of the epithelium are crowded.[5] Most of the tumors show more or less of each of these types of growth, one type generally predominating. Sometimes one part of a section is composed solely of one type and another part of another type. Sometimes these various structures are

[4] This corresponds well with the figures given for human solid ovarian tumors by Massabuau and Etienne (19), who found that of 250 such tumors 43 per cent were bilateral. Of the eight cases of ovarian tumors in mice reported by Jobling, Tyzzer, and Haaland, three were bilateral.

[5] Such a type of growth is shown by Tyzzer (17) in his figure 24.

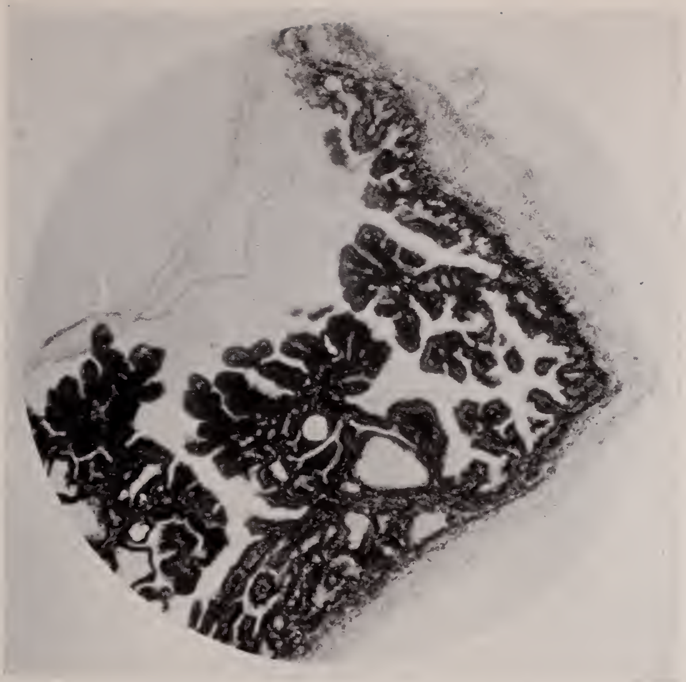

Fig. 7. Papillary Cystadenoma

The only specimen in our series typically reproducing this type common in the human ovary. Mouse 12111. × 45.

Fig. 8. Solid Teratoma

Shows in this field islands of cartilage, epithelial plugs with hornified epithelium, cysts lined with squamous and with columnar epithelium, and varying types of stroma elements. Mouse 9278. × 60.

limited by a distinct basement membrane of true stroma cells, but often there seems to be a false stroma of crowded, spindle-shaped epithelial cells without sharp demarcation from the true stroma cells that may be present. Usually the total amount of stroma is small, most of the growth being composed of epithelial cells, and this stroma is of the cellular ovarian type and not ordinary fibrous tissue. Not infrequently, however, the stroma elements form the bulk of the growth (fig. 3). Generally the capsule is formed by compressed ovarian tissue, in which ova or follicles are rarely seen. Blood vessels are usually scanty; but, nevertheless, necrosis or other retrogressive change is seldom found. Mitosis is very rarely observed, nor are forms suggestive of amitotic division common. The epithelium resembles the germinal epithelium and occasionally the surface of the tumor is covered with this cuboidal epithelium which dips down at intervals to divide the growth deeply into lobules.

As variations from the structure described above we have in a few instances the formation of several small cysts with lining of flattened epithelium. In a few also the structure suggests a papilloma with all spaces obliterated by pressure or from lack of secretion by the surface epithelium. If the growth is of tubular character the pressure usually causes the lumens to resemble narrow slits (figs. 2 and 6). In some instances the alveolar structure is filled with flattened cells consisting chiefly of deeply staining nuclei, producing a growth resembling the adenomatous growths often found in the human vermiform appendix. In only one instance have we a true fibroadenoma (12922) in which a large part of the tumor is composed of definite fibrous stroma with abundance of collagenous material, rather than the cellular ovarian type of stroma. We have not identified any of our tumors as of lutein cell structure, nor have we observed fibromas or myofibromas in the ovary.

MALIGNANT OVARIAN TUMORS

We have found the following tumors that seem to be unquestionably primary malignant tumors arising in the ovary.

6487. The left ovary was 10 mm. in diameter, nearly spherical, partly white and fleshy, partly cystic. There were no

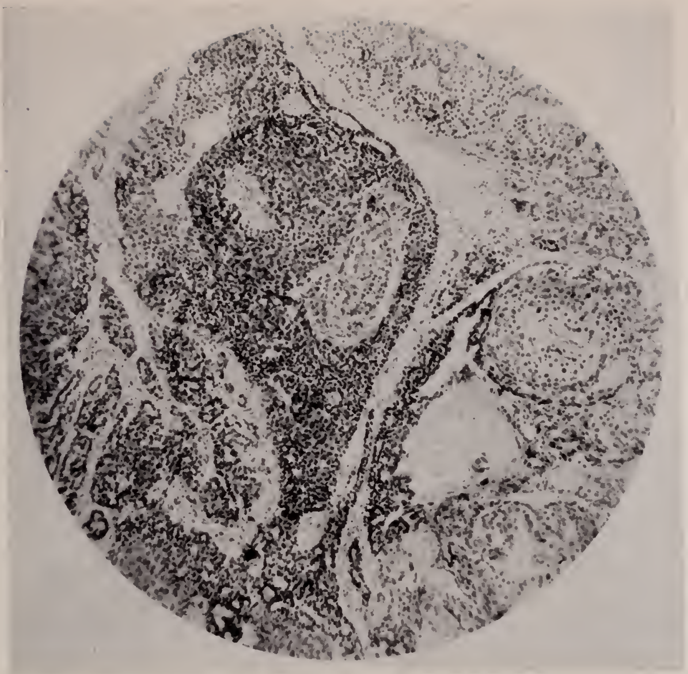

Fig. 9. Malignant Epithelial Tumor of Ovary

Showing both tubular and alveolar types of growth. Mouse 6487. × 100

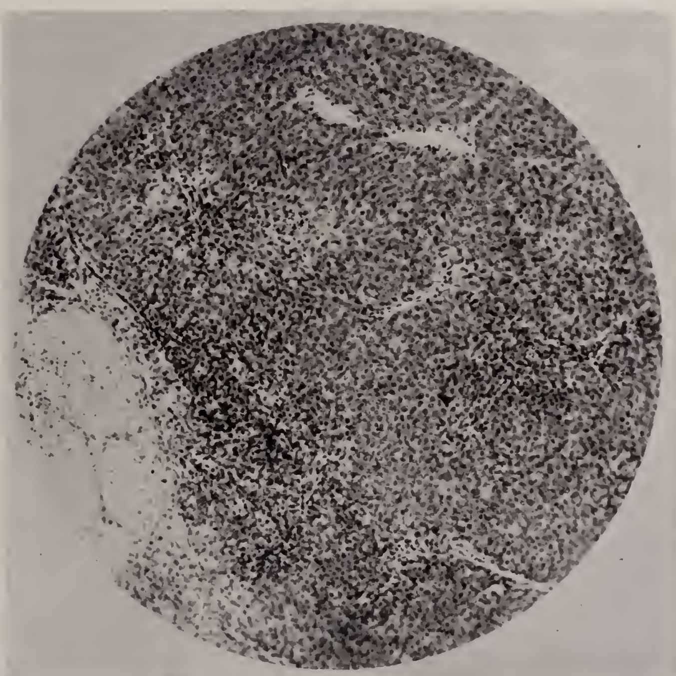

Fig. 10. Malignant Epithelial Tumor of Ovary

Showing solid type of growth of large cells without any well defined arrangement. Similar structure is also shown by tumors of testicle and adrenal. Mouse 12552. × 110.

adhesions and no evidence of metastasis. The mammary gland exhibited a large hemorrhagic carcinoma and also a minute early carcinomatous nodule, both of these growths being typically and unquestionably primary carcinomas of the mammary gland; structurally they were entirely distinct from the ovarian growth. About one half of the latter was composed of a cavity filled with a protein-rich fluid, and with walls formed by an irregular border of tumor tissue. Apparently this cyst was formed by degeneration of tumor tissue, as it had no proper cyst wall, although on the side towards the tumor there are some papillary outgrowths into the cavity. The solid part of the tumor consists of epithelial cells arranged mostly in large irregular alveoli, often exhibiting central necrosis, and occasionally atypical tubules are seen (fig. 9). The stroma is scanty and of ordinary connective tissue type for the most part, although these are areas with abundant elongated cells. The cells are large with a clear cytoplasm, well defined borders, and oval nuclei which are partly solid and partly slightly vesicular. Occasional mitotic figures are seen. Although there are no metastases, the histological structure of this tumor is such that it must be considered to be malignant, an alveolar carcinoma of the ovary.

12552. The left ovary measured 25 by 18 by 18 mm., nodular, with many distended blood-vessels coursing over it. It was not adherent to adjacent tissue and no metastatic growths were found. In the mammary gland were two hemorrhagic carcinomas, entirely different in structure from the ovarian tumor. Microscopically the ovarian tumor is composed of large irregular alveoli filled with large cells with a moderate amount of cytoplasm and a large pale nucleus, with very little stroma (fig. 10). Several mitotic figures were found. The original ovarian capsule surrounds the growth, but no recognizable ovarian tissue remains. Areas of necrosis and hemorrhage are numerous. This tumor closely resembles 6487 but exhibits a greater degree of malignancy.

6801. The left ovary was converted into a hemorrhagic nodular mass, 18 by 15 by 10 mm. The right ovary was normal.

A mass of tumor tissue lay anterior to the left kidney, about the size of the kidney itself. There was a large carcinoma of the mammary gland arising in the left flank, and producing metastases in the lung, as shown by microscopic examination.

The large ovarian tumor consists of about equal parts of extravasations of blood or large dilated blood channels and of tumor tissue. Nothing of the original tissue remains, except that in the capsule appear a few cells suggestive of ovarian stroma elements. The tumor has no well defined structure, consisting of large solid masses of cells which occasionally are formed into trabeculae by blood channels, but otherwise show no tendency toward grouping of any kind. The cells are characterized by a considerable amount of cytoplasm which is usually homogeneous and often with well defined cell borders. The nuclei are round, sometimes solid and sometimes vesicular. Where the cells are largest they resemble slightly liver cell types. There is practically no stroma besides the blood vessels, which are scanty except for the large channels in the tumor without vessel walls. The retroperitoneal mass presents quite the same structure. Mitotic figures are not seen. A few multinucleated cells are found. The general character of the growth resembles that of other malignant tumors found in the adrenal and testicle.

14099. The left ovary is about three times the normal size, and of a red color. The uterus is distended with a milky fluid. In the upper lobe of the right lung is a firm nodule, 2 mm. in diameter, which proved to be a simple papillary adenoma, apparently benign but with a projection growing into a blood-vessel. There were no changes of significance in the other organs. Microscopically the ovary is found to be almost entirely replaced by a mass of large cells with no particular arrangement, which invade the remaining recognizable ovarian tissue, and in which appear epithelial structures that resemble overgrown Pflüger's tubules. The nuclei are deeply stained and irregular in size. In the perirenal tissue is a small growth of similar character.

It is interesting to observe that the malignant types of tumors arising from the ovary, testicle, and adrenal that we have studied exhibit such a similar histological picture, and one quite dis-

similar from tumors arising in other tissues. There can be little doubt that they all represent reversions to the primitive embryonal tissues of the urogenital anlage, and are probably best designated by Adami's term, mesothelioma. This similarity of structure makes it difficult to determine the origin of a tumor involving both the ovary and the adrenal, as in the following case.

12307. The abdominal cavity shows several nodules whose exact origin is difficult to determine as the mate has partly devoured the body. The right ovary is, however, easily distinguished. It measures 18 by 12 by 12 mm. What seems to be the left ovary is 10 by 8 by 6 mm. There are 8 other similar nodules in the abdominal cavity, one being in the position of the left adrenal, measuring 10 by 8 by 8 mm. The other nodules are apparently in the mesentery. One lobe of the liver is converted into a tumor nodule 14 by 10 by 18 mm., irregular and lumpy in outline, pink in color.

The tumor tissue shows everywhere the same structure, consisting of irregular alveoli composed of large cells with abundant cytoplasm with well defined borders and deeply staining nuclei. Mitotic figures are numerous. The character is that usual to mesothelial growths. The ovary cannot be positively identified, but one mass exhibited in the capsule structure suggests compressed ovarian tissue with degenerated ovum. In all respects this tumor is identical with the malignant ovarian tumors just described.

It seems probable that this tumor arose in the ovary which exhibited the largest growth, but it is not possible to exclude the adrenal as the primary site.

Still more difficult to locate is the primary growth in the following case.

12876. The left kidney contains a mass of pink, fleshy tissue, 18 by 14 by 14 mm. The right kidney, which is slightly enlarged, contains no tumor mass. The right ovary consists of a pinkish tissue resembling that in the kidney, and measures 12 by 8 by 8 mm. In the mesentery is a similar, slightly paler mass, 16 by 8 by 8 mm. The retroperitoneal and subcutaneous glands are not enlarged and no nodules are found in the lungs.

Microscopically the tumor tissue is alike in all three places, consisting of a diffuse infiltrating growth of large round cells, which also invade the connective tissues about the kidney and ovary. It does not at all resemble the typical ovarian tumors, being apparently a round-cell sarcoma. We have no way of telling which of the three tumors was primary. The next case presents similar difficulties.

26. This mouse had a tumor mass about 8 by 10 mm. in the upper portion of the liver, with other smaller nodules near it. A similar small nodule was found in the right kidney. The right ovary was enlarged to two-thirds the size of a kidney, and was solid. Microscopically all these growths are composed of round cells, apparently a round-cell sarcoma. It is impossible to say which growth was primary.

We have seen few instances of secondary tumors occurring in the ovary. The Krukenberg tumor is not found because mice do not have gastric or other abdominal carcinomas, except most rarely (20).

In leukemia and pseudoleukemia, infiltration of the ovary with lymphoid elements is common, and often very striking. In our collection there has been no case observed of secondary carcinoma of the ovary, which is not surprising in view of the relatively slight tendency of mouse carcinomas to produce metastasis elsewhere than in the lungs. The following examples of secondary sarcoma have been noted:

12058. A bilateral sarcoma of the uterus, round-cell in type, with metastasis in the right kidney. The left ovary was 10 mm. in diameter and showed complete replacement by sarcoma tissue. As near as we can determine the growth in this case arose in the uterus, and invaded the ovary by infiltration.

19061. A spindle-cell sarcoma growth infiltrated the retroperitoneal tissues extensively, including the pancreas, also with metastasis in the liver. Apparently primary in either the retroperitoneal tissues or in the mesentery. The uterus and both ovaries were infiltrated by the same tissue.

To summarize, we have found four malignant growths that seem to be certainly primary in the ovary. Each of these

exhibited the structure common to malignant tumors arising in the sex glands and adrenal. One exhibited retroperitoneal metastasis near the kidney. Another tumor of similar histological type also involved the adrenal, and produced numerous metastases; in this case it was not possible to determine whether the growth was primary in the ovaries or in the adrenal.

There were two round cell sarcomas involving the ovary as well as other organs, which might have been primary in the ovary, but the evidence did not permit of deciding this. There were two other cases in which the sarcomatous invasion of the ovary seemed to be unquestionably secondary. No secondary carcinomas were observed in the ovary.

TERATOMA OF THE OVARY

From the accounts of animal tumors in the literature it would seem that teratomas are extremely infrequent in the lower mammals. In reviewing the literature on the occurrence of tumors of the ovary and testicle, in the lower animals the chief sites of teratomatous growths, we have found mention of but one case. That was described by Winokuroff (21) as a teratoma of the testicle in a chicken, the growth exhibiting cartilage, bone, striated muscle, squamous epithelium, and cysts. Our own experience supports the view that the lower animals rarely exhibit teratomas, for in the 22,000 mice here considered containing over three thousand spontaneous primary tumors, we have observed but one teratoma. This arose in the ovary, and is a typical example of solid teratoma as shown by the following description:

9278. Death resulted from pulmonary infection. There were no other changes of importance except in the left ovary, which measured 20 by 18 mm., and yielded an exudate from the cut surface. Microscopically the growth has a delicate but distinct capsule in one portion of which there still remains a trace of the original ovarian tissue with one follicle containing an ovum. Except for this the entire section shows a mass of tissues of all sorts thrown together in an entirely disordered

manner (fig. 8). There are numerous small cavities, some of which are lined with squamous epithelium containing chiefly desquamated cells and some polynuclear leukocytes. Occasional solid plugs of squamous epithelium also occur. In places these are branching and occasionally a basal-cell type of growth is seen. No true hair follicles are recognized although occasionally the epithelial downgrowth suggests these structures. No sebaceous glands are found. Most of the squamous-cell structures are grouped together in localized areas. There are also spaces lined with columnar or flattened epithelium, sometimes with a content resembling a diluted mucin. Spaces are found in which part of the lumen is lined with stratified epithelium and part by cuboidal or columnar epithelium. Sometimes these tubules have a well defined coat of non-striated muscle fiber. Small islands of cartilage are abundant, usually having no definite relation to other structures. No bone is seen. Some of the cavities contain old extravasations of blood in varying stages of disintegration. In one place there is a mass of heavy brown pigment near which are collections of small round cells; the whole appearance suggests that possibly this area represents undeveloped retinal tissue. The stroma in general consists of a loose fibrous tissue with abundant cells. There are also many cells with much more cytoplasm than ordinary connective tissue cells, this cytoplasm having a slight basophilic tendency. No striated muscle is found or definite organ tissues but there are many accumulations of cells which are not stroma cells and which presumably are undifferentiated cells of special tissues.

Since this analysis of 22,000 autopsies was made, another instance of teratoma of the ovary has been observed, so that we now have found two teratomas among 25,000 autopsied mice. The chief features of this second case are as follows:

24172. Mouse, eleven months old, died apparently from acute pulmonic infection following delivery, but when found post-mortem changes were too far advanced to be certain of the diagnosis. The puerperal uterus showed no gross evidence of infection. There were no tumors except that involving the right ovary, which measures 30 x 15 x 15 mm., and is nodular,

encapsulated, but not adherent. The mass is soft in consistency and heterogeneous in appearance. There is no exudate or cyst formation on the cut surface.

Microsopically the growth is found to be composed of many different sorts of tissue elements, but unfortunately a large part has undergone so much necrosis and post-mortem change that the structures cannot be well studied. In the portions that do stain the elements are extremely varied and without any particular relation to one another. The greater part of the tissue elements cannot be identified as to their origin or character, being merely masses of cells with small nuclei and considerable cytoplasm. Conspicuous are the plugs of squamous epithelium, with masses of hornified material in the center, but recognizable skin, hair follicles, or sebaceous glands are not found. Tubules lined with columnar epithelium are also occasionally found. There is unusually little tendency to form epithelial-lined cavities or cysts. Numerous areas of mucoid degeneration are present, but goblet cells are not present in these areas, nor can the cellular origin of the mucin be determined. There are a few small islands of bone tissue, but only a few minute groups of cartilage cells. Nonstriated muscle fibers are abundant, usually in distinct bands, but striated muscle fibers are not found. A very little fatty areolar tissue is present. Except for fibrous tissue and blood vessels these are all the tissue elements that can be identified, although some areas resemble liver cells, and others simulate developing nervous tissues. Nothing suggestive of malignancy is found. The diagnosis is clearly solid teratoma of the ovary.

COEXISTENCE OF OVARIAN TUMORS WITH OTHER TUMORS

As emphasized in previous papers of this series, mice with tumors in one tissue exhibit tumors in other organs with a frequency greater than would correspond to the average incidence of tumors. It may be recalled that of the 8 cases of ovarian tumors recorded in the literature, in 4 there were tumors elsewhere in the body.

Among our 40 mice with benign ovarian tumors, 22 had tumors elsewhere, and frequently these were multiple tumors. Of our 4 mice with primary malignant ovarian tumors, all had tumors elsewhere; two mice having each two carcinomas of the mammary gland, one having a single carcinoma of the mammary gland, and one a papillary adenoma of the lung which had invaded a blood-vessel, being therefore presumably malignant. The additional tumors in the 26 mice with both ovarian and other tumors, were located as follows:

Sixteen had one or more primary carcinomas of the mammary gland.

Four had papillary adenoma of the lung.

Two had papillary adenoma of the lung and carcinoma of the mammary gland.

One had adenoma of the mammary gland.

One had a carcinoma and a sarcoma of the mammary gland.

One had a subcutaneous sarcoma.

One had a subcutaneous sarcoma and an osteosarcoma.

SUMMARY

Among 22,000 mice of the Slye stock dying natural deaths at all ages were 44 mice with spontaneous primary ovarian tumors not including simple ovarian cysts. Of these, 38 had simple benign solid papillary adenomas, only occasionally with slight cyst formation. One showed a typical papillary cystoma. One had a typical solid teratoma containing a great diversity of tissue elements.[6] Of the 38 cases of solid papillary adenomas, 19, or 50 per cent, were bilateral, so that there were 57 tumors of this class. There were 4 unquestionably primary malignant tumors of the ovary, all showing the "mesothelioma" type of growth characteristic of malignant tumors derived from the sex glands; one of these produced perirenal metastases. There was one other tumor of the same type primary in either the ovary or the adrenal. Two round-cell sarcomas were found that arose either from the ovary or some other organ, while two other sar-

[6] A second case of teratoma has been observed since this analysis was made.

comas had produced secondary growths in the ovary. Of the 44 mice with primary ovarian tumors, 26 had tumors in other parts of the body.

In the literature were found reports of 8 other cases of tumors arising in the ovaries of mice, which exhibited quite the same characteristics as the tumors described in this paper.

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RHABDOMYOMA OF THE OVARY

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Tumors of striated muscle are sufficiently rare to make the observation of a new and characteristic case worthy of record. The rhabdomyoma which is the subject of the present discussion is of special interest because of the peculiar forms assumed by the myogenic cells and the wide variations of structure in the tumor.

At the present time a detailed discussion of the literature of this neoplasm is unnecessary; but the facts which are of more direct bearing on the present case may be mentioned. Benenati's list of 65 cases was published in 1903; and the cases reported since then do not show any material change in the relative frequency of this tumor in the various parts of the body. They may be divided according to the regions in which they occur. Rhabdomyoma is found most often in the genitourinary tract. There are 39 cases occurring in this region: kidney, 13; testis, 9; uterus, 6; pelvis of the kidney, 3; vagina, 3; bladder, 3; ovary or uterus, 1; ovary, 2. The tumor of the ovary described by Virchow in 1850 was a papillary cystic rhabdomyosarcoma, some of the papillae being formed of striated muscle. A second rhabdomyoma of the ovary was reported by Vignard. It was similar to the tumor about to be described, the greater part being striated muscular tissue with cystic degeneration at one extremity.

Wolfensberger noted the frequency of this tumor in the neck and adjoining regions, which stand second to the genitourinary tract with 9 cases. These localities are: orbit, 2; temporal bone, nose, tongue, parotid, mandible, esophagus, and mediastinum. Four examples are found posterior to the pelvis. They were in the lumbar region, hip, ischial tuberosity, and anus.

Homologous rhabdomyomas are found with greatest frequency in the heart, where 8 cases are recorded. Recently Wolbach has added an instructive case to the list. The remaining 5 examples were found in the following regions: pectoralis major, breast, shoulder, elbow, and thigh.

Muller has recently published a case in which rhabdomyosarcoma followed successive fractures of the femur. In his case the tumor probably arose from previously normal adult voluntary striated muscle cells. Such tumors, while probably not infrequent, are rarely reported, and belong to a group entirely different from the ordinary heterologous or teratomatous rhabdomyoma.

Clinically, rhabdomyomas are tumors of moderately rapid growth. Most of the tumors are well encapsulated and form no metastases; however, there are others with infiltration of surrounding tissues and recurrence after removal (Billroth, 2 cases; Buhl, Kaschewarowa) and some which formed metastases (Wolfensberger, Eberth, Benenati). Burgess has reported a case in which there were multiple metastases throughout the body.

Cohnheim's theory of embryonal rests has been accepted by many authors as accounting for the genesis of this tumor, especially of the heterologous rhabdomyomas. Ribbert has suggested as the origin of his renal tumor the development of striated muscle from the smooth muscle present in the pelvis of the kidney. His arguments do not seem to be convincing.

Benenati gives five possible derivations for rhabdomyoma testis. It may arise from smooth or striated muscle derived from the cremasteric muscle or from the gubernaculum; he finally concludes that the new growth arises from an embryonal rest. In 1904, Ribbert came to the conclusion that rhabdomyoma testis is a one-sided development of a teratoma. In the present communication I hope to extend this interpretation to rhabdomyoma of the ovary.

The genesis of teratoma has been variously explained. Verneuil in 1855 agreed with other writers in finding the origin of this tumor in a twin inclusion. Theories which found the source of tridermal neoplasms in polar bodies and isolated blas-

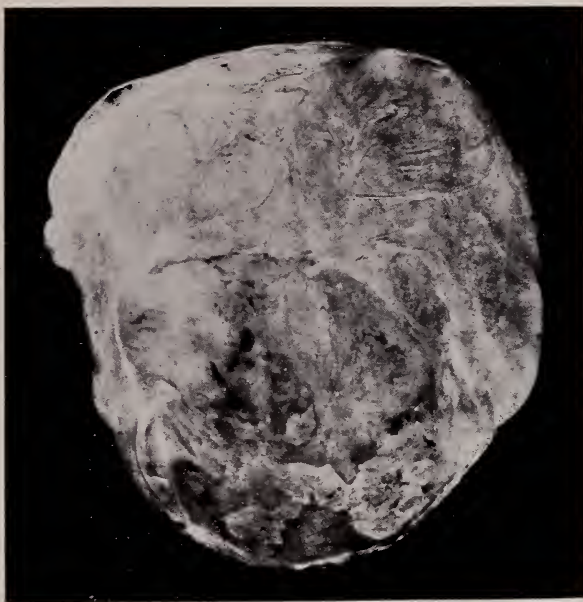

Fig. 1. Gross Section of Tumor

Note capsule and thin strands of connective tissue surrounding the individual spheroidal masses which make up the tumor.

Fig. 2. Low Power Field, Including the Cell Shown in Greater Detail in Figure 5

The giant cells are surrounded by myxomatous tissue. In large portions of the neoplasm the latter element is found exclusive of any other.

tomeres have given way to one which is based on the parthenogenetic development of the sex-cell. At the present time the last mentioned theory has the greatest number of adherents.

The results of experiments performed by Stockard have led me to believe that the twin inclusion theory will best explain the origin of this neoplasm. In the following I shall endeavor to prove that this theory is applicable not only to rhabdomyoma of the ovary, but to most rhabdomyomas in common with many simple tumors occurring inferiorly, either in front or behind the pelvic zones, in the genitourinary tract, or superiorly, in the neck region.

Present case. October, 1918, Edith M., one and a half years of age was found to have a mass in the abdomen reaching half way to the umbilicus. In shape and size it seemed to resemble a kidney with the long diameter horizontal. It was freely movable. There were no subjective symptoms. At the time of the operation nine months later, the mass appeared to fill the whole abdomen up to the umbilicus. Early in February, 1919, the child begun to vomit to such an extent as to cause the mother to give consent to operation. At that time a yellowish discoloration appeared on the skin about the umbilicus.

Operation, performed February 9, 1919, disclosed a well encapsulated tumor arising apparently from the region of the left ovary, filling the pelvis, with a narrower upper portion lying under the inferior surface of the liver. The capsule was attached to the anterior abdominal wall posterior to the umbilicus. The entire new growth was removed. In the process of removing the tumor the capsule was broken so that some of the myxomatous tissue fell into the abdominal cavity. The child died of abdominal recurrence, May 31, 1919.

Gross anatomy. The tumor is covered by a thin, movable capsule and consists of two parts, a lower, hard, spherical mass, 11 cm. in diameter, and an upper myxomatous portion, 4 cm. in diameter. It weighs 26 ounces. The surface is smooth and presents various rounded protuberances. On section of the larger part, the capsule is seen to run inward from the surface in thin strands throughout the tumor, giving the impression that it was the covering of an ever increasing growth. The tumor is made up of rounded masses, 3 cm. to a few millimeters in diameter, suggesting that it grew by the appearance of new parts, as well as by the increase in size of the older portions. On section the myxomatous division was found to contain a cavity.

Fig. 3. Giant Cell with Extension of Fibrillated Process in Muscle Fiber Formation

Adjoining are muscle fibers showing to some degree longitudinal and cross striation.

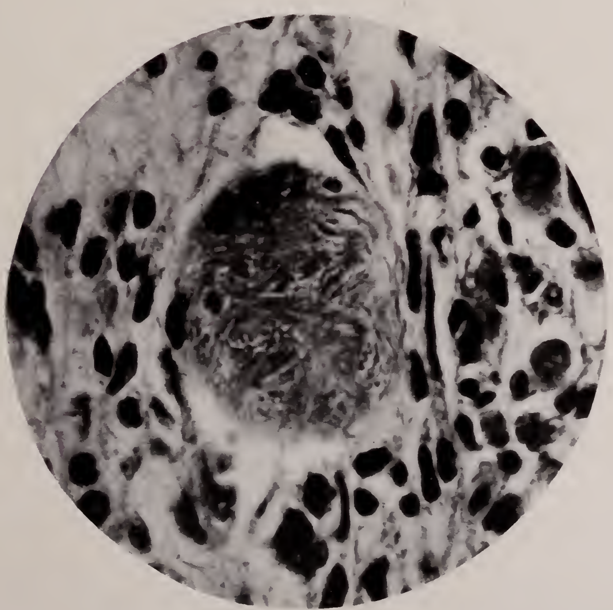

Fig. 4. Giant Cell with Well-Formed Fibrils Arranged without Definite Order

There are four nuclei situated at one end of the cell

Structure. Several main histological features deserve description. Perhaps the most striking are the *giant cells.* These cells have an acidophile cytoplasm in which usually there are concentric striae, most prominent at the periphery, while the perinuclear cytoplasm is granular. The nucleus or nuclei are round or broadly oval, sometimes containing 1 to 3 nucleoli. In other cells one or more centrosomes, as shown in figure 6, give evidence of active mitosis.

Muscle fibers. There are areas in the tumor composed of long cells with the characteristics of muscle fibers. The nuclei are oval and usually situated in the median axis. The peripheral cytoplasm is fibrillated, but only rarely can any cross striations be made out. Some of the fibers branch.

Myxomatous tissue. In several parts star-shaped cells with nuclei the size of those seen in the muscle fibers, and fibrils extending radially from them, can be seen separating the muscle fibers, showing that they developed alongside each other. In other places, they are the only element seen in large areas, giving the tumor a distinctly myxomatous appearance.

Histogenesis. The normal embryology of muscle may be taken as a guide to the anaplastic process. Striated fibers arise from myoblasts which elongate and by repeated mitotic division of their nuclei form a syncytium. The nuclei are surrounded by granular cytoplasm in which fibrils differentiate peripherally. The myofibrils became striated. The fibers increase in size and the nuclei migrate to the periphery. Heart muscle undergoes a similar development; but the nuclei remain centrally situated, sarcolemma never develops, and the individual syncytia are in connection with each other. Assuming that the tumor arose in a cell or group of similar cells we might expect to be able to follow the development till we arrive at the picture given in the histological description. Examining the three elements of the tumor it is possible to discern such an unfolding.

Giant cells. The giant cells as a group show a fundamental error in development; they have failed to elongate.

1. There are giant cells which show the extension of a process in an abortive development of muscle fiber (fig. 3).

2. There are giant cells which never elongated, while in other respects, they went through a more or less normal and complete development (fig. 5).

3. There are others which show increasing anaplasia until we come to some in which the fibrils lack definite order (fig. 4).

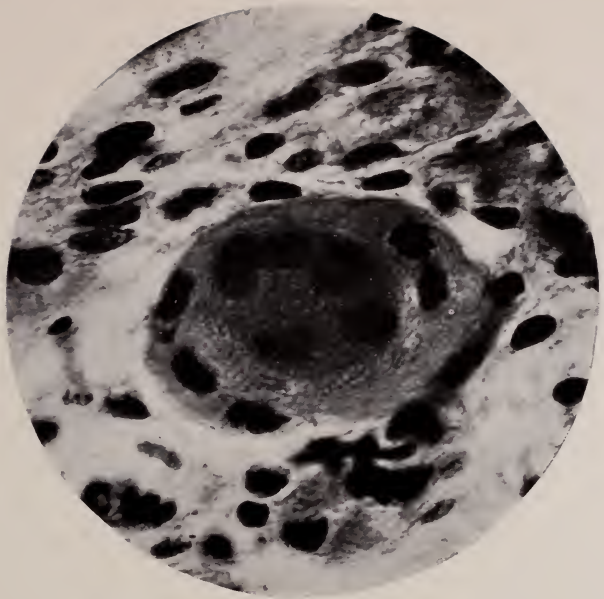

Fig. 5. This Cell Did Not Elongate, but in Other Respects It Went Through a Complete Differentiation

The fibrils and cross striae are shown as concentric and radial striae

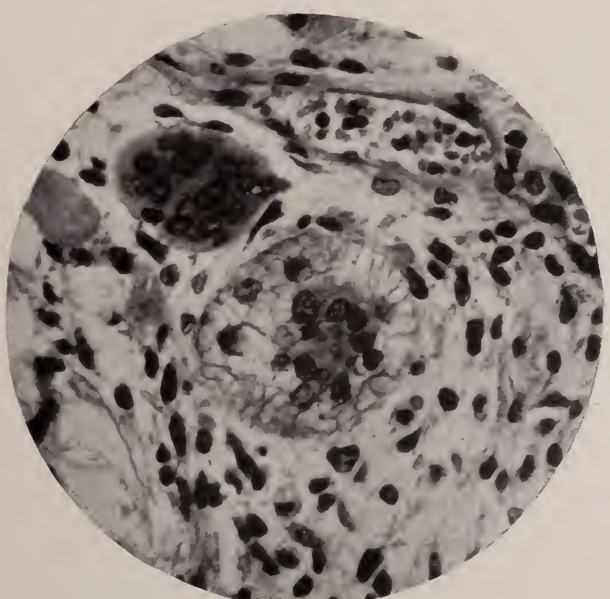

Fig. 6. "Spider Cell" in which the Cytoplasm between the Periphery and the Nuclei Has Degenerated and Been Absorbed

The perinuclear cytoplasm contains five centrosomes. This cell occurs only in cardiac rhabdomyoma.

I am indebted to Mr. William Dunn for the photographs.

4. Lastly, there is a group of cells, some of which resemble the "spider-cells," first described by Cesaris-Demel in a remarkable tumor, a rhabdomyoma of the heart (fig. 6). Others lie in spaces which Wolbach showed to be intracellular. Seiffert concluded that these spaces were formed within the peripheral differentiated portion of the cell by the degeneration and disappearance of the greater part of the remainder of the cytoplasm. The histogenesis of the "spider-cells" is similarly explained.

The fact that this type of cell has been found exclusively in rhabdomyoma of the heart, and the fact that I have found branching in some of the fibers, have led me to conclude that the present tumor arose from heart muscle. Katsurada, among others, reported heart muscle in dermoid cysts. It should be added that while sarcolemma is occasionally developed in rhabdomyomas, it is never found in those of the heart. Also, here as in heart muscle, the perinuclear cytoplasm remains undifferentiated. Some writers have described branching in other rhabdomyomas. This may indicate that in an anaplastic process such forms may be evolved, or that these other neoplasms had an origin similar to the one now being described.

The second element of the tumor need not long detain us. The muscle fibers are descendants of cells which developed more normally than those heretofore mentioned.

There are many cells which show intermediate stages of development between the muscle fibers and myxoma cells. The genesis of the myxoma is explained as follows: it occurred in the most rapidly growing portion of the tumor, and, as is well known, parallel with an increasing rapidity in the multiplication of cells there is a corresponding loss in differentiation.

In this laboratory there are three other rhabdomyomas with a mucous element very similar to the one described. The literature affords other cases in which myxoma complicated an otherwise pure rhabdomyoma (Billroth, Kaschewarowa, Targett). The possibility thus arises that with the preponderance of myxomatous tissue a rhabdomyoma may be misinterpreted as a pure myxoma.

In some parts of the tumor surrounded by myxomatous tissue, the nuclei disappear, leaving areas of pure fibrils. These areas of fibrils raise the question of the possibility of the multiplication of fibrils without the corresponding multiplication of the myxoma cells. An analogous condition seems to obtain in neurocytoma, in which a similar picture of excessive fibrils may appear. It does not seem possible for the multiplication of fibrils to take place without the corresponding division of nuclei, but the overgrowth of fibrils of the individual cells is quite possible. A similar overgrowth of cytoplasmic derivatives is seen in the formation of epithelial horn.

Origin of the tumor. 1. Having shown that all the elements may be derived from myogenic cells, the next question to be considered is the origin of the group of cells from which the tumor arose. In considering this heterologous neoplasm we may leave a metaplastic origin out of the question. This leaves two possibilities: the tumor arose (a) from an embryonal rest; or (b) as a one-sided teratoma.

We may safely eliminate the former possibility. In general, nothing is known about embryonal muscle rests in the ovary. In particular, the organ is not situated near striped muscle so as to make such a rest possible. This conclusion becomes more evident since we are dealing with cardiac muscle.

It is an accepted fact that one element of a teratoma may outgrow the others (Pick and Walthard) and that the neoplasm may be represented by a single tissue. In the case of rhabdomyoma this conclusion gains added strength when we remember that striated muscle sometimes identified as cardiac, is often found in teratomas. It is interesting to note that Cornil thought the rhabdomyoma described by Vignard, the only case in the literature similar to the present one, was a fetal inclusion.

Additional support is found in the occurrence of similar tumors in an analogous organ. Rhabdomyoma testis is admittedly of teratomatous origin.

2. Since the teratomatous origin is the only one which will explain the rhabdomyoma it is necessary to determine in what manner the teratoma arose, and again there are two possibilities; it arose either from a sex cell or from a twin inclusion.

In the ovary, where there are totipotent sex cells, it may seem unnecessary to seek further for the origin of the teratoma. The commonly accepted explanation is the parthenogenetic development of this cell. This view point is open to criticism because mammalian ova, with the exception of one series reported by L. Loeb, have never been observed to develop parthenogenetically and then continue a separate existence. Loeb, himself, claims little vitality for these ova, while Bandler and Wendeler quote other investigators to the effect that ova which undergo such a development invariably end in complete degeneration.

On the other hand, the twin inclusion theory has a basis in experiments recently performed by Stockard. By slowing the rate of development of fish embryos at the time when the primary bud should arise, two or more buds may develop simultaneously. The degrees of doubleness of the resulting individual is determined by the distance between the two buds on the blastodisc. At 180°, that is diametrically opposite each other, two complete individuals are formed.

When one bud gets the start of the other by any advantage it obtains a supremacy which allows it to develop into a perfect individual. The component arising from the inferior bud is suppressed and interfered with so that it develops abnormally. The twins are attached anteriorly in the head and neck region, or posteriorly, the great majority of them in the latter situation. There are all degrees of this inhibition of the second twin until it is reduced to a mere fragment of included tissue. The point made by Stockard is that human monsters have been born which show exactly the same relation to each other, as above described. Monsters attached superiorly on the head, neck, or upper thorax, or inferiorly near the sacrum, or anteriorly in the ventral region, can be traced in an unbroken series until the inhibited twin is represented by a teratoma or dermoid.

On the basis of these experiments it is possible to explain the presence and frequency of rhabdomyomas in the regions given in the first part of the paper, that is, 9 superiorly in the neck region, 43 inferiorly in the pelvic region.

No facts have been brought forth to disprove the twin inclusion theory. However, there are two questions which need further discussion. The first is the great frequency of teratomas in the sex glands; and the second, the occurrence of chorioma in males and in females at such a time as to shut out the possibility of impregnation. These phenomena can be explained by the twin inclusion theory.

Bonnet remarked the fact, in defending the theory of the origin of teratoma from an independent blastomere, that if a blastomere should separate from the remainder of the blastula, the organ to which it would be most likely to attach is the mesonephros.

For a long time the mesonephros is the largest organ in the abdominal cavity. It is the most vascular. Later the mesonephros survives as functional and vestigial parts of the genital system; in the male, the efferent ducts, the paradidymis, epididymis and vas; in the female the paroöphoron, parovarium, and Gartner's ducts.

If the twin were attached to the mesonephros, we should expect to find it adherent in the testis to the mesonephric tubules which join those of the sex gland. This is actually the case, for Ewing in his paper on teratoma testis points out that almost invariably the neoplasm arises in the neighborhood of the rete. We can explain those which occur in the scrotum external to the tunica albuginea by the accidental attachment to other portions of the mesonephros. Bandler has made an attempt to explain teratoma as a development of the paroöphoron. We now see that while it does arise from this region it is not a development from this organ, but is rather attached to it.

The position of teratoma is explained by the organogenesis of the sex glands in which the mesonephros plays such a prominent part. This accounts for most teratomas attached anterior to the pelvis. It explains the 39 rhabdomyomas found in the genitourinary system. The other four, being situated posteriorly, were not able to become adherent to the mesonephros. From these facts arises the thought that many simple tumors of the ovary may be of teratomatous origin. Ewing has come to

the conclusion that practically all common tumors of the testis are of similar origin.

In taking up the question of chorioma, we may again turn to an observed fact. Up to the present time nobody has been able to cause the spermatozoa of any species to develop into an embryo. How then can we explain chorioma testis except as an early inhibited twin with the predominance of this single chorionic tissue?

Boestrom reported a case of chorioma with multiple metastases throughout the body in which the testicles were normal. Djewitski recorded a case of chorioma of the bladder in a virgin, seventy-five years old. Surely rather late for the parthenogenetic development of an ovum, but not against the growth of a long repressed twin. The facts of extragenital chorioma can be explained best as a twin inclusion. The relations between the two components is such that with the growth of one there is an inhibition of the other. The rate of development of the host after birth is much retarded, and the repressed component is given the required opportunity to grow. This explains why the majority of teratomas begin their growth in early life rather than during the longer adult period. Senile atrophy of the host gives the parasite a last chance for development.

Causal genesis. The frequency of teratoma testis after trauma is well known. This may not only directly precipitate the rapid growth, but indirectly, by lowering the vitality of the organ, give the long repressed twin inclusion the temporary advantage. The real causal genesis lies in the potential energy for growth constantly waiting a chance for expression. Further, that the growth should be autonomous is the only possibility in tissues which have been so disorganized. This may be capable of experimental verification.

To sum up the points of the theory of the twin inclusion: It is based on observed facts and therefore can give a true casual genesis. It explains the position and frequency of teratomas, in the sex glands as well as elsewhere in the body, obviating the necessity for multiple explanations for teratomas. The theory holds for all one-sided teratomas.

The possibility of new evidence in favor of any other explanation for the frequency of teratomas in the sex glands will not disprove the twin inclusion theory, but will be complementary to it. Likewise the theory holds independently of the method of the formation of the twin.

According to the theory of twin inclusion, the complete sequence of events is as follows: an ovum is fertilized; through some unfavorable condition the rate of development is slowed; two primary embryonic buds are formed on the blastodisc, one having the advantage over the other. The favored bud develops into a perfect individual, during which process the smaller bud is inhibited and disorganized. The latter, being united to the more vigorous component at its ventral region, becomes adherent to the mesonephros; hence, later it forms a part of the ovary. With the birth of the child and its consequent diminished rate of development, the inhibited twin is given a new opportunity to grow. Through its previous disorganization it is not capable of orderly development. The heart muscle grows more rapidly and succeeds in crowding out the other elements. The growth gains in momentum, and myxoma cells are formed which have no resemblance to the earlier muscle cells.

SUMMARY

The case described is one of rhabdomyoma of the ovary in an infant.

Three elements arose from one tissue by (a) histogenesis more or less normal; (b) anaplastic development; (c) degenerative changes. Although only one tissue, cardiac muscle, is present, the tumor is of teratomatous origin. Many simple tumors of the ovary are probably of similar origin. Many tumors of the head, neck, thorax, and genitourinary and posterior pelvic regions are of teratomatous origin. This group includes the great majority of heterologous and some of the homologous neoplasms.

Teratoma is a twin inclusion. A group of cells is found in this tumor which appears only in rhabdomyoma of the heart.

Therefore, the present tumor is a rhabdomyoma of the heart of a twin inclusion. Fibrils may be produced in such overabundance as to lose connection with their nuclei and seem to be multiplying independently. Myxoma may be secondary to rhabdomyoma.

I wish to thank Dr. Stockard and Dr. Ewing for help in the preparation of this paper.

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MALIGNANCY OF THE CROWN-GALL AND ITS ANALOGY TO ANIMAL CANCER[1]

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METHODS EMPLOYED IN THE INVESTIGATIONS

In 1907, Dr. E. F. Smith showed that the disease of plants called crown-gall—tumor-like in nature—could be reproduced artificially by an inoculation of a normal plant with a pure culture of a bacillus which he called Bacterium tumefaciens. Smith maintains that this or a similar disease can be produced only by this particular organism, that all other tumor-like formations observed in plants due to other parasites, like Plasmodiophora brassicae, are entirely different pathological entities, and that only crown-gall is a true plant tumor. In his estimation, this tumor is practically identical, biologically, with animal and human cancer. Since crown-gall is caused by the action of the bacterium described by him, he concludes in one of his recent articles that "to a biologist the conclusion is almost irresistible that human cancer must be due to a parasite and that one parasite may well be the cause of the most diverse forms, as we have seen to be the case in plants." This deduction is so sweeping in character, so far-reaching in its generalization, and the results of Dr. Smith's work have become so widely known and quoted by the medical profession that it has seemed to the writers to be desirable to renew his investigations, to analyze the material from the viewpoint of animal pathology, and to attempt to determine the true analogy between the crown-gall and animal cancer.

[1] Presented before the American Association for Cancer Research, June 14, 1919.

The writers have inoculated with the pure culture of Bacterium tumefaciens the following plants: Growing tips, stems, petioles, and blades of Rosa (Rose), Ricinus (castor oil), Pelargonium (house geranium), Lycopersicum (tomato), Nicotianum (tobacco), Helianthus (sun-flower), Bryophyllum calycinum and Ficus elastica (India-rubber tree). Galls formed were fixed at various stages of development in Carnoy, Flemming strong,

Fig. 1. Crown-Gall on a Stem of a Rubber Plant

Hermann, Merkle, and Bouin's solutions. The first fixation was found best suited for the more woody galls. After embedding in paraffin, the sections were cut from 5 to 30 micra in thickness, and were stained with Flemming's triple stain, methyl green counterstained with fuchsin, or Delafield's hematoxylin counterstained with eosin. The most satisfactory preparations were obtained by fixing with Carnoy solution and staining with gentian violet and safranin. In these preparations the cell walls stain a

beautiful deep violet, while the cytoplasm and chromatin material stain a much fainter purple. The nucleolus takes a bright ruby red color.

FIG. 2. *a*, CROWN-GALL ON A STEM OF A ROSE; WART-LIKE BENIGN GALL. *b*, LONGITUDINAL SECTION OF THE SAME STEM

The gross study of the material shows that in nearly every inoculated plant there developed a distinct crown-gall within one or two months after inoculation. Usually these galls grow slowly and are not very large in comparison with the size

Fig. 3. Longitudinal Section of a Stem of Ricinus Plant
The gall is benign, the normal stem tissue is only displaced

of the plant itself. Figure 1 shows a gall which grew for over six months on a rubber plant; the plant itself is 6 feet high. Most frequently the gall grows out of the surface of the stem and does

FIG. 4. *a*, RICINUS PLANT INOCULATED AT THE APICAL PORTION OF THE STEM; SHOWS DWARFING OF THE PLANT. *b*, CONTROL PLANT

not seem to affect the rest of the plant (fig. 2). In other instances the growth displaces and distorts a part of the cortical area of the stem (fig. 3). Only in a small percentage of the inoculated

plants does the crown-gall affect the tissues of the plant more deeply. Figure 4 shows a photograph of the growing regions of two plants, one inoculated with the microorganism, and the other a normal control plant. While the latter grew up above the tip,

FIG. 5. PELARGONIUM
Shows necrosis of the inoculated part of the plant

the inoculated plant ceased to grow and became dwarfed, and the leaves drooped. At the same time, even in the inoculated plant, the lower part of the stem as well as the root apparently remained normal. Figure 5 presents an instance where the part of the plant above the point of inoculation, and even to a certain

degree below the point of inoculation, became necrotic. Cross (fig. 6) or longitudinal (fig. 7) sections of such plants show that these crown galls grow invasively, destroy and replace normal

Fig. 6. Longitudinal Sections of Two Stems of Ricinus Plants
Show invasive growth of the gall and destruction of the normal stem tissue

plant tissue, and occasionally develop further along the length of the stem in a manner which produces the appearance of a secondary metastatic tumor above or below the primary growth (fig. 8).

In accordance with the findings of Smith, the writers have also observed in many instances the formation of small leafy shoots at the periphery of a crown-gall (figs. 9 and 10).

The microscopical analysis of the material shows that the crown-gall consists of a number of uniformly small, young, undifferentiated cells which contain a greater amount of cytoplasm

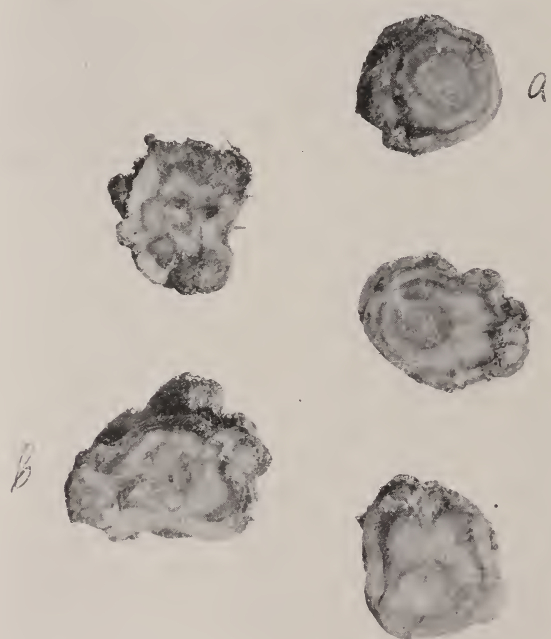

Fig. 7. Cross Section Showing the Gradually Increasing Invasion of the Normal Stem

The invasion is least at *a* and greatest at *b*

than the normal parenchyma cells within which they develop (fig. 11). The nuclei frequently show mitosis.

The small slowly growing crown-galls which frequently develop when the inoculation is done at the bases of leaves (fig. 12) present a very striking picture when examined microscopically. In the center of the growth there is an area of characteristic crown-gall cells, and surrounding this area the crown-gall cells appear

to be much larger in size and present all the characteristics of normal parenchyma cells (fig. 13). Apparently in these crown-galls the peripheral gall cells became differentiated into adult

Fig. 8. Longitudinal Sections Showing at *a* and *b* Conditions Somewhat Similar to Metastasis Formation

normal tissue cells. Frequently there were observed new irregular conducting systems formed within the crown-gall tissue (fig. 14), also apparently as a further differentiation of the crown-gall cells.

Microscopical study of a large number of the leafy sheets which developed at the periphery of a crown-gall showed that the starting point and the base of each leafy shoot is ordinary crown-gall

FIG. 9. LONGITUDINAL SECTION OF A STEM OF A TOBACCO PLANT

The apical portion of the stem was removed and the cut surface inoculated. The crown-gall shows the development of leafy shoots.

tissue with the identical gall cells (fig. 15), indicating that the crown-gall cells themselves gave rise to the formation of the leafy shoot.

DISCUSSION

The analysis of the material shows that crown-gall is undoubtedly a *neoplastic disease* and that the pathogenesis of the condition consists in an abnormal proliferation of a group of cells.

FIG. 10. TOBACCO LEAF INOCULATED AT THE MID RIB
Shows at *a* a small gall with a leafy shoot

It is self-evident, therefore, that there must exist many points of analogy between the crown-gall and animal cancer. Since a

plant presents a much less complex organization than the animal, crown-gall tissue undoubtedly offers an ideal material for the study of the phenomena connected with cell proliferation. All this does not imply that crown-gall or any other neoplastic disease in animal or plant must of necessity be a tumor, even less so a malignant tumor—a cancer.

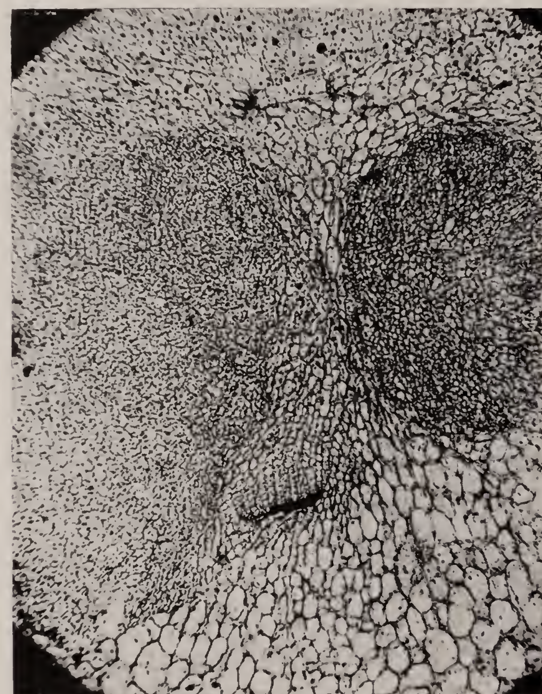

FIG. 11. MICROPHOTOGRAPH
Shows crown-gall tissue invading the normal stem tissue

In comparing new growth in animals and plants one must take into consideration the fact that an adult vertebrate is not capable of reproducing complete organs, while the highest plants may and do constantly reproduce with ease all their organs, leaves and branches as well as roots. Reproduction of parts of the organism, and consequently cell proliferation, is a function of an adult plant which may be induced with the greatest of ease.

It is self-evident that since an adult plant cell proliferates so as to reproduce an organ, a young crown-gall cell may do likewise. This characteristic of a normal plant cell explains the striking

FIG. 12. FOUR LEAVES OF RICINUS INOCULATED AT THE UPPER PORTION OF PETIOLES
Shows small wart-like galls

phenomenon, not encountered in any neoplastic disease in the animal, that a crown-gall may form within its own cells, or rather

as a transformation of its cells, not only adult differentiated tissues (parenchyma) but even rudimentary organs (conducting system) or a whole rudimentary organism (leafy shoot). All these various types of structures found in a crown-gall do not indicate, as appears to be the opinion of Smith, that the crown-gall is analogous to all the types of human cancer, but rather that it is different from any type of animal tumor.

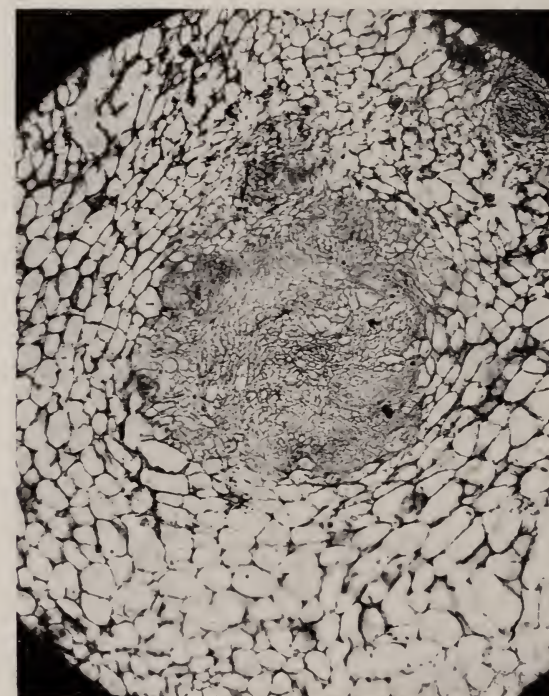

Fig. 13. Microphotograph of a Gall Shown in Figure 12

The characteristic gall tissue is surrounded by newly formed parenchyma tissue.

In order to formulate clearly the position which the crown-gall occupies among the neoplastic diseases, one must take into consideration the fact that the crown-gall is usually a benign condition and only rarely does it act in a manner analogous to a malignant tumor in an animal.

The true mechanism of the formation of a benign crown-gall can be conceived only in the light of the difference in the structure of an animal and a plant organism. An animal organism reacts frequently to an attack of any injurious agent with the so-called inflammatory processes, which are accompanied by certain activities of the lymphoid tissues and proliferation and migrations of lymph cells. These cells, and not the special tissues of the injured region, offer protection, envelop and destroy the injurious

Fig. 14. Microphotograph
Crown-gall showing newly formed irregular conducting system

agent, or neutralize it. When the latter is destroyed, the inflammatory process ceases and functions of repair or replacement of the injured tissue take place. This repair, which ends in the formation of a scar, is a type of neoplasia which differs radically from tumor formation. It continues just as long as is necessary to replace the lost tissue and then ceases; while tumor formation is a neoplasia which has no reason for its formation in the needs of the organism, and has no finality in its development.

The highest organized plants do not possess any specialized lymphoid tissue to take care of the functions of the protection of the organism against an injury or of the repair of the injured or lost tissue. One of the methods of protection of plants against injury consists in the presence of a cellulose wall. It seems plausible to suppose that as a matter of self-protection a plant may respond to an injury by a proliferation of the cells of the

FIG. 15. MICROPHOTOGRAPH
At *a* crown-gall tissue, and at *b* the tissue of a leafy shoot

region which was subjected to the injury. The small wart-like galls, which grow very rapidly for a time and then remain stationary, as the writers have observed in a number of instances, are products of such functions of repair and protection and do not represent true tumors. That the Bacterium tumefaciens may be found even within the crown-gall cells or in the immediate neighborhood, still further emphasizes the truth of this conception.

Thus a small benign crown-gall is a condition analogous to granulation tissue and a scar in the animal organism. A crown-gall which grew to comparatively large size and still does not affect in any way the general welfare of the plant may be compared to a benign tumor in an animal, but is probably more nearly analogous to a large callus, which develops after a fracture of a long bone, or to a cheloid in the human.

Smith ascribes a great deal of importance to the phenomenon of development of leafy shoots in a certain number of crown-galls, and considers this type of gall to be identical with human embryoma. This finding he considers the main proof for his contention that all types of human cancer may be reproduced in plants by the aid of the Bacterium tumefaciens. In the opinion of the writers there is no analogy between a human embryoma and the crown-gall with a leafy shoot sprouting from it. An embryoma is a growth consisting of an irregular combination of various fetal tissues. It is more akin to a malformation than to a true tumor. A malignant tumor may develop subsequently within an embryoma in the same manner as it develops within normal tissue. The leafy shoot on the other hand appears on a fully developed crown-gall and is identical with a shoot which develops in a normal part of a plant. It simply indicates that crown-gall tissue as well as normal plant tissue may reproduce complete organs and thus still more widens the gap between the animal tumors and the crown-gall.

The malignant type of crown-gall is undoubtedly quite analogous to animal cancer. This condition occurs very rarely, as is seen from the results of the field studies on sugar beets of Townsend, who found that the destruction of the beets by the gall is not sufficient to influence the tonnage of the crop.

The most enthusiastic supporters of the parasitic theory of cancer admit that the formation of benign tumors cannot be due to a parasite. In other words, they admit that the mechanism of the formation of benign and malignant tumors must differ from each other. The same is undoubtedly true as regards the formation of the usual benign type of crown-gall and the by far less frequent condition when the gall dwarfs or destroys the plants

and thus acts as a malignant tumor. When two plants of the same size, the same age, growing in the same soil under identical conditions of heat, light, moisture and nutrition, are inoculated in the same regions with the same quantity of an identical culture of Bacterium tumefaciens, and there develops in one plant a small, benign, wart-like structure and in the other a large malignant tumor which may destroy the plant, it is difficult to conceive that the same microorganism, and only the microorganism, created the two conditions. The only possible explanation of the phenomenon lies in the fact that in the second plant, for some unknown reason, the cell proliferation which began only as a protection against the bacterial invasion and apparently ceased when the bacteria were rendered harmless, suddenly received an impetus for limitless proliferation. It seems then that the first impetus to the cell proliferation and formation of a benign crown-gall may be caused by the Bacterium tumefaciens. But the transformation of this protective or inflammatory benign cell proliferation into a malignant tumor is due, as in every type of animal and human cancer, to some mechanism within the organism of the host, independent of the microorganisms, the nature of which is unknown.

CONCLUSION

1. Crown-gall is a neoplastic disease, and offers an ideal material for the cytological study of cancer.
2. Crown-gall occurs both as a benign and a malignant condition.
3. The benign crown-gall is analogous to granuloma or cheloid in the human and is caused by Bacterium tumefaciens.
4. The malignant crown-gall is analogous to animal cancer, and Bacterium tumefaciens is not the direct cause of the malignant transformation.

ON THE KINETIC AND INVASIVE POWER OF REGENERATING TISSUE AND ON SIMILARITIES IN THE BEHAVIOR OF THYROID TRANSPLANTS AND CARCINOMAS

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The following observations are of interest because they clearly demonstrate that some of the fundamental characteristics of cancerous growth can be found in regenerative growth. In addition, they bring out very sharply some of the factors which, under ordinary conditions, restrain regenerative proliferation.

I. INVASION OF BLOOD VESSELS BY REGENERATING THYROID TISSUE

In the course of a study of the factors which determine regeneration of the thyroid gland and the fate of thyroid transplants, we compared autotransplantation of one thyroid lobe in cases in which the second lobe had been left intact and in which it had been extirpated previous to the transplantation. We also compared the fate of autotransplants and homoiotransplants at various stages.

In an animal, a female guinea-pig (no. 758) weighing 370 grams, we transplanted one lobe of the thyroid subcutaneously into the same animal. The lobe of the other side was left intact. Seven days later the transplant was taken out for examination. The recovered piece was relatively large and cut into serial sections. Microscopic examination showed the following:

A connective tissue capsule rich in fibroblasts surrounds the piece; it encircles a ring of acini, which, however, is not complete, being interrupted at such places where fat tissue constituted the periphery of the transplant. The peripheral acini are the larg-

est ones; they contain often well formed colloid. Other acini are filled with blood and a few contain some polynuclear leucocytes. Towards the center the acini become smaller, and especially is their lumen diminished. At various places the inner acini seem to end in cell strands without lumen. These cell strands accompanied by growing fibroblasts grow towards the center of the piece, which latter consists in the main of thyroid tissue which, being deprived of blood supply after transplantation, had become necrotic and in part hemorrhagic. In the necrotic walls of the central necrotic acini some disintegrating polynuclear leucocytes are seen. In one small area of the necrotic center a somewhat larger collection of disintegrating polynuclear leucocytes can be observed. The acini as well as the more centrally situated epithelial strands contain relatively frequent mitoses. In the peripheral concentrically arranged capsule of connective tissue we find some thyroid tissue in the form of extensive ducts; they may send off branches in various directions. On the whole they follow the direction of the connective tissue capsule in which they are embedded. These ducts are perhaps acini which regenerated in the connective tissue capsule, and under the influence of the growing connective tissue assumed a concentric growth similar to that of the connective tissue itself. Mitoses are also found in these ducts. They are situated in the periphery of the ring of acini. There is on the other hand a possibility that they took their origin in epithelial ducts which occur even in the normal thyroid.

In the periphery of the transplant and inside of the connective tissue capsule we find running parallel with the capsule a large vessel. It had been transplanted with the thyroid and may perhaps have partly recovered after transplantation. It consists of two layers, an inner endothelial and an outer muscle layer. It is situated at a place where the thyroid ring is interrupted by fat tissue. This vessel gives off branches which enter septa of the transplanted thyroid and begin to take a course radially towards the center.

At one place the vessels connect with a large capillary which, accompanied by fibroblasts, traverses the greater part of the

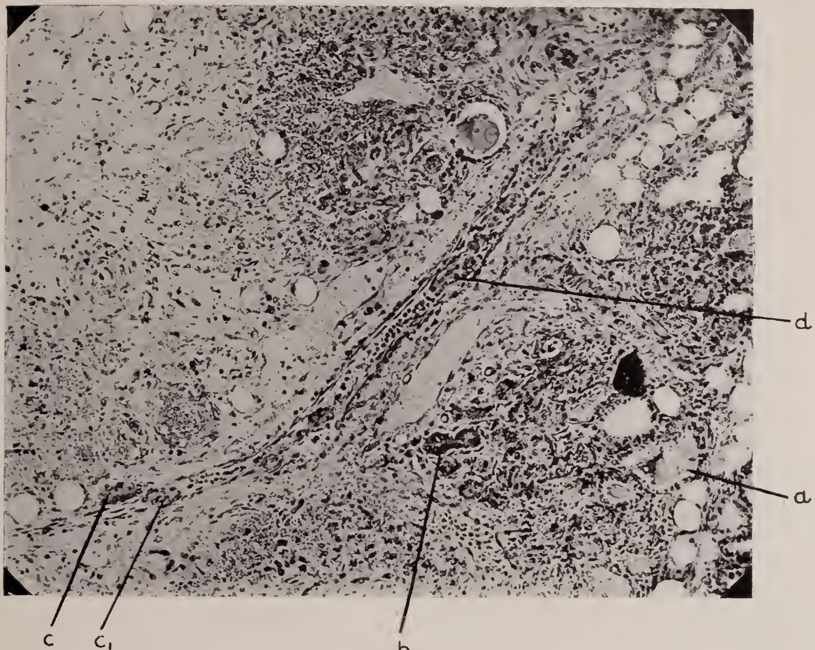

FIG. 1

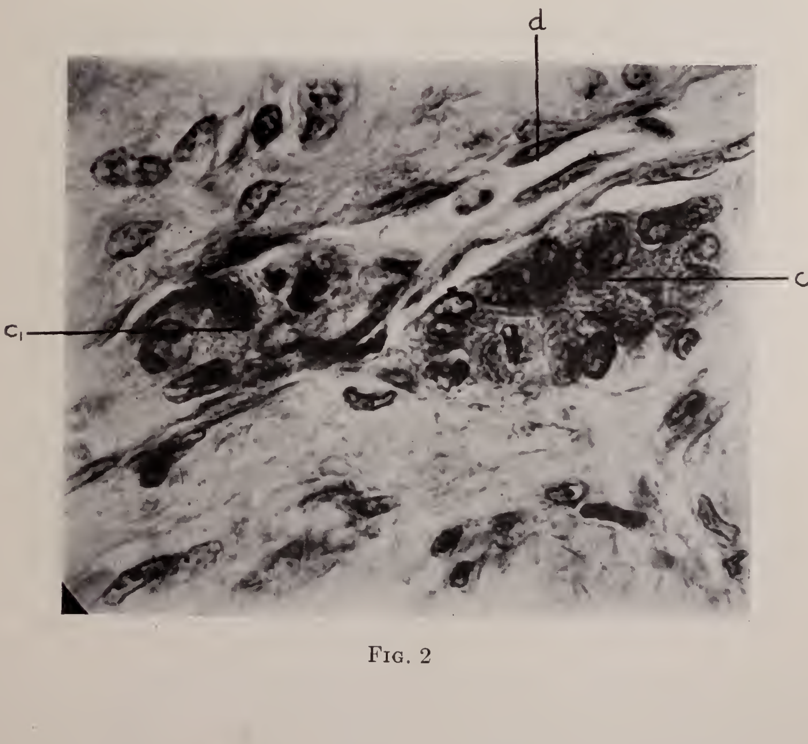

FIG. 2

necrotic center of the thyroid and here ends as narrow capillary tubes. This capillary again sends off branches at various places. There is actual growth taking place in these vessels, as evidenced by the presence of mitoses in the endothelial cells. It is furthermore probable that the coat of unstriated muscle cells which surrounds the larger vessels gradually extends towards the periphery, certain pictures suggesting that migration of cells may play a part in this growth process.

Fibroblasts accompany the growing vessels and organize the necrotic tissue which the vessels traverse. These fibroblasts gradually invade more and more the necrotic center and would in time have organized it.

At several places solid strands of thyroid tissue which are arranged in a radial centripetal direction grow towards the end ramifications of the capillary vessel. At two places they come in close contact with it. At one of these two places we see parts of the thyroid strands outside of the vessel but in close approximation to it. At one place such a strand breaks through the capillary wall and enters the lumen of the vessel (figs. 1 and 2). And from here on one can follow in serial sections how these strands of thyroid tissue fill the greater part of the lumen of the capillary, then enter the large peripheral vessel which we described above (fig. 3) and which connects with the capillary; at last they enter into the various branches of this vessel (fig. 4). Altogether considerable masses of newly formed thyroid tissue fill these vessels. Active proliferation continues in these strands within the blood vessels. We could find several mitoses in the thyroid cells. While in the capillary the thyroid tissue forms thin strands (fig. 1), in the larger vessels it is present in wider aggregates of tissue in accordance with the greater width of the vessels at the periphery of the thyroid (figs. 3 and 4).

While near the place of entrance into the capillary vessel the strands of thyroid tissue are lying free in the lumen, a little higher up they are covered by a layer of endothelial cells which are derived from the endothelium of the vessel. Wherever the thyroid strands are in touch with the vessel wall, endothelium begins to migrate over the strange tissue; thus in the course of time a cer-

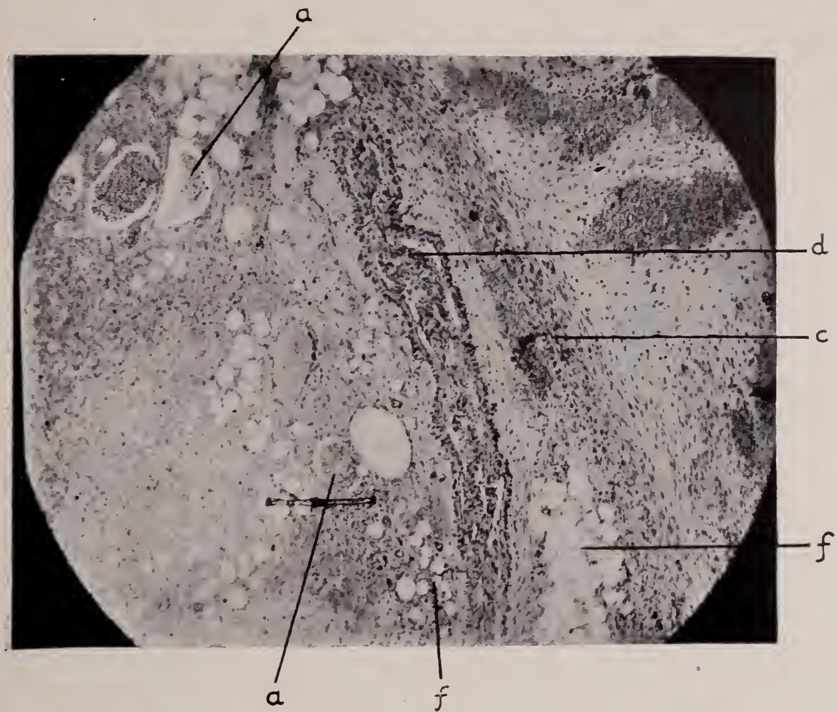

Fig. 3

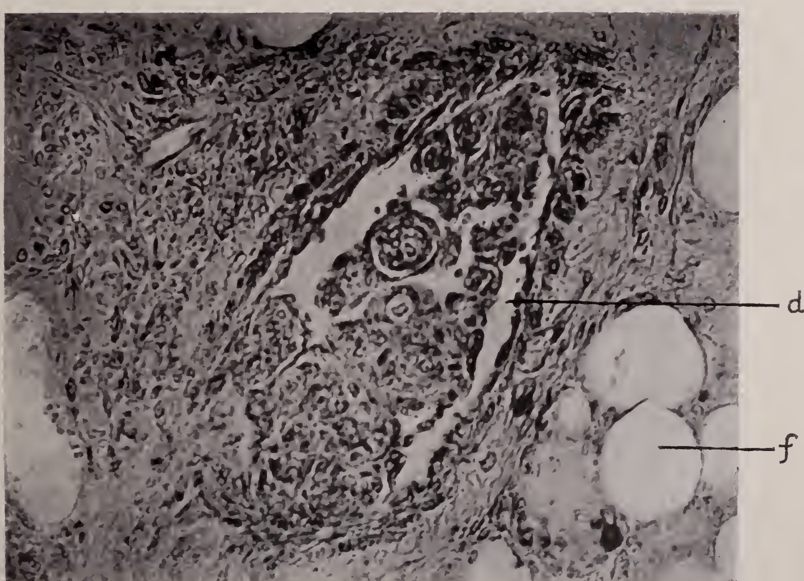

Fig. 4

tain inhibiting effect will be exerted upon the invading tissue by the endothelium of the vessel wall. This inhibiting influence is however as yet unable to prevent mitotic proliferation. More towards the periphery of the transplant within the larger vessel, the thyroid strands form irregular gyrations which are all covered by an endothelial coat. Between these strands of thyroid tissue within the larger vessels spaces are left in which the blood is able to circulate. We see here considerable masses of well preserved blood corpuscles. Further toward the center of the transplant in the end piece of the capillary we find mainly polynuclear leucocytes. Here evidently stagnation in the blood current occurred and in consequence of this the white corpuscles collected in larger numbers. A similar aggregation of polynuclear leucocytes occurs, whenever the circulation is slowing down at a certain place.

We must assume that at least temporarily the invading tissue has come to a standstill within the vessel; otherwise an investment with endothelium would not have been possible; nevertheless it is very probable that a shifting of the tissue in a peripheral direction continued to take place as a result of mitotic proliferation and perhaps also of continued invasion in the capillary in the center of the transplant. Furthermore considering the presence of circulating blood within the larger vessel a breaking off of certain parts of the thyroid strands is a possibility. Such broken off tissue might be carried into capillary vessels of distant organs and perhaps remain alive for a certain period of time.

II. INVASIVE AND GROWTH POWER OF REGENERATING AND CARCINOMATOUS TISSUE

These observations are of general interest in the first place because they demonstrate the marked similarity between regenerating and cancerous tissue, and secondly because they prove that the potentiality of forming new tissue inherent in regenerating tissue is in many cases considerably greater than could be assumed from the amount of tissue which is actually formed.

Cancerous tissue shows an increase in proliferating energy over the normal tissue from which it is derived; it likewise very often shows an increase in ameboid activity. As the result of both these processes an invasion of the surrounding tissue takes place as well as an invasion of blood and lymph vessels with subsequent formation of metastases. The stimulus acting on the cancerous cells may be very strong or the condition under which the stimulus reaches the cells may be abnormal. These abnormalities find expression in certain morphological changes which we often find in cancerous growth, such as a simplification in structure, irregularity in mitoses and in the structure and size of nucleus and cell and amitotic division of nuclei.

None of these changes are however entirely characteristic of cancer. An increase in proliferative energy and ameboid activity is found in regenerating tissue. We have formerly shown (1) that regenerating tissue may likewise show an increase in its power of infiltrating neighboring tissue especially after the latter has become less resistant. Thus we saw regenerating tissue entering blood clots, coagulated blood serum, and agar, and branching within these media in various directions. We found it breaking through cartilage and surrounding particles of cartilage and carrying them along on their migration. We may presume that in this case the cartilage had been previously injured, perhaps through a temporary infiltration with leucocytes. It is known that a simplification of structure is often observed in regenerating tissue and under certain conditions morphological abnormalities may be found in regenerating tissue similar to those observed in cancerous tissue.

We now can add to these characteristics the invasion of blood vessels on the part of regenerating tissues and at least the possibility that such invading tissue may become detached from its connections with the surrounding tissue and be carried through the blood stream to a distant organ. Whether here a real metastasis could ever be formed under these conditions appears doubtful considering the transitory character of the increase in proliferative energy which is characteristic of regenerating cells. The possibility can, however, not be denied that under certain

conditions an additional stimulus, such as might perhaps be exerted by the abnormal environment, in which the transferred cells find themselves, may cause a retention of their increased growth energy and ameboid activity at least for a certain period of time, and that thus a metastasis consisting of apparently normal thyroid tissue may occur. Metastases of apparently not cancerous thyroid tissue have been noticed by various observers.

In a number of cases it has been observed that particles of apparently normal parenchyma entered the blood vessels and were retained in small blood vessels of the lung. Thus particles of bone marrow, liver cells and chorionic villi have been found (cf. O. Lubarsch (5)). There can be little doubt that in such cases we have to deal with a mechanical misplacement of tissue usually due to trauma of some kind. In no case is such an embolism the result of an active invasion of blood vessels such as we have observed.

The difference between regenerating and cancerous tissue is then mainly one which is quantitative as far as the intensity as well as the duration of changes are concerned, in cancer the increase lasting considerably longer than the stimulus and often becoming at least potentially perpetual or eternal. As a result of the action of proliferative stimuli a new cell equilibrium has been established in which through inner cell mechanisms those proliferative stimuli are furnished which originally had been called forth by external stimulation.

We see then that cancerous as well as regenerating cells are stimulated cells and, both show therefore similar reactions. The differences between regenerating and cancerous cells are mainly of a quantitative character as far as the energy and time factor are concerned and we must assume that in the cancer cells secondary inner regulations have taken place which are absent in the normal regenerating cells.

Our observations indicate that the amount of tissue which is actually produced during regeneration is considerably less than the amount which could be produced if certain restricting influences were absent. Thus we find that of the numerous cell strands which form at various places at the inner circumference

of the thyroid ring only those situated at one or perhaps two places are able to produce large amounts of tissues, viz., those situated in close approximation to growing blood capillaries. They break into the vessels and encountering in the lumen of the vessel less resistance than outside, they extend over a considerable area partly through mitotic proliferation and in all probability also through cell movements. If other cell strands had been situated equally favorably near growing capillaries, they likewise might have penetrated into the lumen of the vessels and produced much new tissue. Then the total amount of newly formed tissue would have greatly exceeded the amount actually produced.

At all other places the strands found mechanical obstacles to their proliferation and extension. The necrotic and hemorrhagic material filling the center of the transplant and at some places perhaps also fibrous tissue opposed the epithelial growth. The expansive energy of the regenerating epithelium can evidently be neutralized by a certain amount of resistance which depends upon the mechanical properties of the environment. It is certain that the expansive energy of carcinomatous tissue being lastingly increased would be able to overcome a resistance which proves too strong in the case of regenerating tissue. Moreover in many cases the expansive power of carcinomatous tissue is not only extending over a longer period of time but is also more intensive. This is, however, not necessarily so in every instance. Thus we found in a case of multiple carcinoma of the skin in a young man the corium offering so strong a resistance to the expansive tendency of the cancerous tissue that at one place the latter was forced to proliferate towards the outside of the skin (2).

III. INVASION OF MUSCLE AND FAT TISSUE BY REGENERATING THYROID

These observations concerning the kinetic and invasive power of regenerating thyroid tissue do not represent exceptional or isolated occurrences. Thus we have found another transplant in which the regenerating acini contained in their lumen particles

of fat tissue. They evidently had surrounded cells of fat tissue which were situated near them and closed around them to form acini.

Very frequently remnants of epithelial ducts or perhaps also peripheral acini which are situated in the growing fibroblastic tissue proliferate with the latter and form extraordinarily large epithelial spaces, which run parallel to the connective tissue. They may branch in various directions towards the central thyroid tissue as well as towards the surrounding proliferating connective tissue, and often multiple and large papillae are produced in these ducts as the result of the fibroblastic proliferation. We have mentioned such a duct in the first thyroid which we described in this paper.

In another case in which a thyroid had been removed seven days following homoiotransplantation (guinea-pig 670) we saw growing acini running parallel to proliferating connective tissue and advancing towards the outside and infiltrating the muscle tissue of the host; during this process the acini were accompanied by growing connective tissue and capillaries. At one place the lumen of an acinus contained a piece of the muscle tissue which had been surrounded by the growing thyroid strands and included in the acinus in a similar way to the fat tissue mentioned above. Many mitoses were found in this proliferating thyroid tissue.

IV. ACINAR AND ALVEOLAR TYPE OF GROWTH IN THYROID AND CARCINOMA

There is another similarity between the regenerating thyroid and certain carcinomata of the mammary gland in mice which however is perhaps in part at least more apparent than real. It is a very common occurrence that such carcinomata are structurally of a mixed character and of special frequency is a combination of acini in certain areas of the carcinoma and of solid alveoli in others. The alveoli often show a marked mitotic activity and they may thus enlarge; but it can be shown that there is still another mode in which alveoli grow; the apparently simple alveoli are in many cases merely conglomerations of cell strands, which

form gyrate structures. Gradually as the result of the pressure, exerted by the epithelium, intervening areas of connective tissue become smaller, form a more or less homogeneous mass and ultimately disappear, and thus we have in the end to deal with apparently homogeneous alveoli, whose mode of origin from cell strands is no longer discernible.

In the regenerating thyroid several authors mention a formation of structures not unlike cell nests consisting of squamous epithelium. It is assumed that such cell nests are derived from proliferating acini. If this interpretation were correct, we had in this case to deal with a process analogous to what apparently occurs in carcinoma, viz., a transition from acinar to alveolar structures. Our observations make it however very probable that such a transition is not the mode or at least not the only one in which these alveolar structures originate. We found in the normal thyroid of the guinea-pig ducts which may branch and are surrounded by dense fibrous tissue. Even in the not transplanted thyroid such ducts may occasionally produce solid cell nests. The production of such cell nests from ducts seems to be a frequent occurrence after transplantation of the thyroid. The ducts accompanied by proliferating fibroblastic tissue grow actively and produce a very widely spread, branching network of solid strands, all connected with each other. The connective tissue around them becomes fibrillar and fibrous. In several cases we have been able to trace such a network of alveoli towards a central duct surrounded by dense fibrous tissue, such as we find in the normal thyroid. There can be very little doubt that in these cases the ducts and not the acini give origin to the alveolar structures. The process is analogous to what I have observed in the transplantation of mammary gland where also similar alveolar structures occur, and where they do not owe their origin to a transformation of gland acini, but to a proliferation of the excretory ducts of the gland. Thus all these alveoli are connected with each other.

It is possible that the alveolar structures found in the mammary carcinomata may also be derived from gland ducts rather than from acini. If this should be so, the similarity between the be-

havior of regenerating thyroid and certain carcinomata would be great. If on the other hand the alveoli of the mammary carcinoma are derived from acini, then the similarity would be in part at least more apparent than real. However at present we do not wish to exclude the possibility that the cell strands which take their origin in thyroid acini may also be able to produce solid alveolar instead of acinar structures under certain conditions. Thus in the lumen of the blood vessels the proliferating cell strands, which we described above, proliferate as solid strands and not as acini. It seems however probable that in most cases the typical network of large alveoli sometimes showing approximation to pearl formation is derived from ducts and not from acini.

In another respect however a certain similarity between the alveoli of mammary carcinoma in mice and the alveoli in the regenerating thyroid may exist. The former, as we have seen, may be composite structures. The same may hold good in the case of thyroid alveoli. Solid cell strands growing from different directions towards each other meet, include at first parts of the stroma, which later disappears. This condition was for instance observed in guinea-pig 499 in which a homoiotransplantation of a lobe of thyroid had been carried out eight days previously. It was a rather young guinea-pig, weighing 427 grams. There was found in this case a noticeable infiltration with lymphocytes and much hemorrhage in the center which was perhaps in part due to the rupture of newly formed capillaries. In the center we found solid alveoli. In serial sections it was seen that all or at least the majority of these strands were connected with each other (fig. 5). There is marked mitotic proliferation in these cells. This contributes to the enlargement of the alveoli, but in addition it appears that at certain places neighboring cell strands approach each other, and exert pressure on the intervening stroma and that thus processes of solution take place in the connective tissue. At first one may still be able to discern connective tissue strands within the apparently homogeneous alveoli but ultimately the strands disappear and then it is no longer possible to recognize the origin of these conglomerate

alveoli. Simultaneously with these changes in the epithelial structures changes take place in the accompanying stroma. The fibroblasts form fibrillae and often dense fibrous tissue around these strands and alveoli and it may be that the changes from fibroblastic to fibrous tissue are in part responsible for the lessened nourishment and secondary changes in the center of the alveoli which correspond to similar changes which take place in

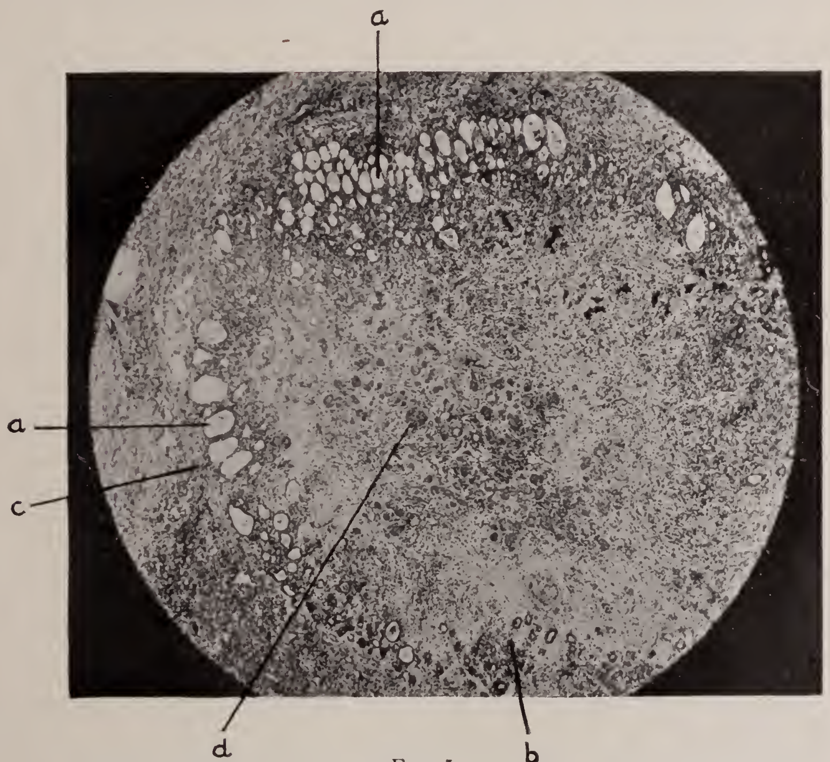

Fig 5

the epidermis whenever it is further removed from the source of nourishment.

These solid cell strands grow especially towards the center and not within the peripheral ring of acini. This is evidently due to the fact that the fibroblasts grow from the peripheral ring towards the center of unorganized material which they organize. It is the movement of the fibroblasts which determines the direction in which the epithelial structures move.

These cell strands cannot under any circumstances succeed in reestablishing the normal thyroid structure. They will always remain epithelial tissue dissociated from neighboring normal tissue; they represent "cell nests." And yet growth within them ceases and they do not produce carcinoma. There is therefore one essential difference between regenerating and carcinomatous tissue. In the latter the growth stimulus continues to act independently of the environment in which the growing cells are, as long as the cells obtain sufficient nourishment; in regenerating tissue the stimulus leads to growth processes which can be represented by a curve which earlier or later approaches the base line, although normal conditions may not yet have been reestablished.

V. ON THE INTERACTION BETWEEN GROWING GLAND TISSUE AND FIBROBLASTS

In the cases mentioned above there can be little doubt that it it is the growing fibroblastic tissue which causes proliferation of the thyroid tissue and at the same time determines the direction in which the epithelium proliferates and moves. It determines the shape of the thyroid cell strands, and the degree and direction of their proliferation in the center of the transplant. It likewise determines the proliferation and the structure of the concentric canals in the periphery of the thyroid and the secondary formation of papillae within these canals. In all those cases the proliferating fibroblastic tissue does not only not prevent, but even stimulates the epithelial growth; at the same time it influences the direction in which it takes place. In accordance with these observations on the beneficial effect of fibroblastic proliferation on epithelial growth we often notice that after transplantation of an epithelial tissue the most active proliferation takes place in that part of the graft which is in contact with actively moving and proliferating fibroblasts of the host, while that part of the transplant which is at a place more distant from proliferating fibroblasts is often not exhibiting any noticeable growth. In the latter case, however, we cannot be quite certain which is the

primary process, the fibroblastic proliferation stimulating the epithelial growth in the graft, or the epithelial growth attracting and stimulating the fibroblasts. On the other hand in the observations to which we referred above there can be no doubt that the fibroblastic tissue is the primary factor.

While thus it is certain that fibroblasts may through their activity stimulate epithelial growth, the reverse effect also occurs. We have formerly described the difference in the character of the stroma in resting and in active mammary gland of the guinea-pig (3). In this case there is no doubt that the epithelial activity is the primary factor which stimulates secondarily the connective tissue. Growing epithelial tissue usually stimulates the surrounding stroma. A similar stimulating effect may be exerted by the growing parenchyma on the blood vessels. In growing carcinoma the proliferation of the epithelial structures quite commonly stimulates the activity of the fibroblasts and of the blood vessels.

While thus the activity of epithelial tissue stimulates fibroblastic and vascular activity in the area adjoining the epithelium, the latter possesses, as we have pointed out previously, the power to prevent the penetration of fibroblasts into the epithelial structure and the epithelial tissue may even actively restrict the fibroblastic growth (4). It seems that it is the "autosubstance" produced in the epithelium which has this power in the highest degree and that it is partly lacking, whenever homoiosubstances are produced through the interaction of transplant and body fluids of the host.

The interaction between epithelial and fibroblastic tissue is therefore evidently complex. Epithelial activity stimulates fibroblastic activity and fibroblastic activity may under certain conditions stimulate epithelial activity. The epithelium possesses mechanisms through which it restrains the invasive power of connective tissue. Under certain conditions epithelium loses to some extent this power (for instance, under the influence of homoiotoxins) and then the connective tissue exerts a destructive influence upon the epithelium. Fibrous tissue restrains the activity of the epithelium and metabolically inactive or pathologically functioning epithelium may favor the transformation of the surrounding fibroblastic into fibrous tissue.

SUMMARY

1. Regenerating tissue of the thyroid may possess an invasive power not unlike that of cancerous tissue. Strands of regenerating thyroid can invade blood vessels and advance and proliferate within their lumen. They may also invade fat and muscle tissue and include in the lumen of the acini particles of such tissue. This invasive power is less intense than that exhibited by very active carcinomata.

2. Growing transplants of thyroid tissue may apparently show a transition from acinar to alveolar structure. Similar transitions can be found in the case of mammary carcinoma in mice. In both cases the formation of alveoli may be due to a conglomeration of cell strands. At first parts of stroma are still included between the epithelial constituents. Secondarily these become dissolved and disappear. It can be shown that in many cases the alveolar network in the center of the transplanted thyroid does not take its origin in acini, but in ducts included in the thyroid. In a similar way alveolar structures in transplants of the mammary gland originate in ducts.

3. The kinetic and invasive activity of regenerating thyroid tissue is associated with fibroblastic activity. There are indications that under certain conditions epithelium and connective tissue exert a mutually stimulating influence, while under other conditions their effect upon each other is antagonistic.

4. The power of regenerating tissue to proliferate is much greater than could be foreseen from the amount of tissue which is actually produced. Usually the environment exerts a restraining influence upon the regenerating activity of the transplant.

5. In contradistinction to carcinomatous growth regenerating growth of the thyroid comes to a standstill, although the typical structures of the thyroid may not yet have been reëstablished and the cell strands are as yet without their normal connections with neighboring epithelium.

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THE GLYCEMIC REACTION IN ITS RELATION TO TRANSPLANTABLE MALIGNANT TUMORS

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That hyperglycemia of a temporary character follows the injection of a relatively large variety of substances has been shown by the results recorded in another publication (1). The relation of this type of hyperglycemia to transplantable malignant tumors in animals forms the subject of the present paper. In the publication to which reference has been made, the observation that a hyperglycemia followed the injection of peptone was confirmed. Three types of reactions were described as occurring after such injections. It was demonstrated that there was no constancy about the reaction type, irrespective as to whether the same or different substances were used in repeated injections.

The first experiment recorded in the present paper has to do with the constancy of the reaction type after the growth of transplanted tumors.

A series of rats was injected with 0.125 cc. of a 1 per cent solution of peptone in distilled water. Immediately before injection and again forty-five and one hundred and twenty minutes thereafter, blood sugar estimations were made according to the Epstein (2) method, blood being obtained from the tail vein.[1] The animals were then inoculated with 0.003 gram of either the Flexner-Jobling carcinoma or the Jensen sarcoma. After twenty-one days of tumor growth the animals were again tested with peptone as before inoculation. As controls, a small series of

[1] In the four animal experiments, all blood sugar determinations were made after starving the animals for twelve hours.

TABLE 1

All figures indicate milligrams of sugar per 100 cc. of blood

	Before				After			
	Zero hour	45 minutes	120 minutes	Type	Zero hour	45 minutes	120 minutes	Type
Flexner-Jobling carcinoma; tumor growing....	180	220	248	I	152	176	160	II
	140	204	212	I	168	174	200	I
	122	120	110	III	140	244	164	II
	110	208	188	II	120	182	168	II
	176	168	200	III	136	110	130	III
	186	158	160	III	152	162	160	II
	108	120	150	I	110	120	124	I
	124	130	142	I	114	110	134	III
	124	144	170	I	130	142	162	I
	138	228	200	II	120	124	130	I
	130	136	134	II	130	160	200	I
	130	140	178	I	140	144	189	I
	156	174	200	I	130	164	108	II
Flexner-Jobling carcinoma; tumor not growing	190	186	174	II	134	130	146	III
	222	176	110	III	110	152	140	II
	124	130	160	I	128	104	162	I
	116	200	124	II	138	164	200	I
	140	132	140	III	134	120	132	III
	170	140	180	III	180	210	160	II
	100	108	136	I	154	178	164	II
	132	178	130	II	110	140	176	I
Jensen sarcoma; tumor growing	102	198	150	II	120	160	168	I
	240	194	168	III	124	148	180	I
	156	150	114	III	130	136	150	I
	140	126	142	III	160	130	138	III
	144	146	134	II	144	190	186	II
Jensen sarcoma; tumor not growing	146	170	184	I	138	130	136	III
	156	122	114	III	170	172	128	II
	150	144	140	III	126	104	100	III
	130	160	116	II	132	146	132	II
Fetal skin emulsion......	102	90	110	III	110	140	142	I
	116	100	86	III	120	160	154	II
	138	100	180	III	112	159	154	II

animals were tested with peptone as were the other animals, and then injected with 0.05 gram of emulsified fetal rat skin. These animals were also retested with peptone twenty-one days after the injection of the fetal skin.

The data of this experiment, which are given in table 1, show that, as was to be expected, no constant type of reaction occurred before inoculation with tumor or injection with fetal skin, and that twenty-one days after the introduction of these cells, the same conditions of affairs existed, irrespective as to the fate of the transplanted graft.

Other experiments carried out concurrently with those described in this paper having indicated that the hyperglycemia following the injection of substances other than glucose was probably related to the processes of immunity, our next experiments were planned so as to use hyperglycemia as an indicator of the development of antibodies, in order to see if hyperglycemia, when interpreted in this fashion, would follow the laws so far determined of immunity against transplanted animal tumors.

It is generally accepted that immunity to transplanted tumors can be induced by the injection of homologous living cells at least ten days before tumor inoculation. It is also generally accepted that autologous or heterologous living cells do not have this power (3).

In one experiment, rat fetal skin was emulsified and extracted for three hours in distilled water. Then 0.125 cc. of this extract was injected subcutaneously into a series of normal mice, spontaneous tumor-bearing mice, and mice bearing the transplanted Crocker Fund mouse carcinoma no. 11. As in our rat experiments, blood sugar estimations were made just before, and again forty-five and one hundred and twenty minutes after injection. The data given in table 2 show that rat fetal skin, representative of an heterologous protein, was capable of inducing a temporary hyperglycemia in all three groups. This result is comparable to the antibody production which follows the injection of any foreign protein, in that while a foreign protein induces antibody formation when injected, it also provokes a hyperglycemia.

In another experiment, the effect of injections of autologous protein extracts was studied. The spleens were removed from a group of rats, this operation having been shown in our first paper not to interfere with the hyperglycemic reaction. The spleens were emulsified and extracted in their own bulk of distilled water for three hours. As before, sugar estimations were

TABLE 2

Effect of injections of heterologous protein extracts. All animals injected with 0.125 cc. of rat fetal skin extract. All figures indicate milligrams of sugar per 100 cc.

	ZERO HOUR	45 MINUTES	120 MINUTES
Normal mice	140	152	118
	170	140	110
	150	192	215
	128	135	165
	117	128	175
	131	190	110
Mice bearing growing mouse carcinoma 11	136	130	158
	147	156	120
	110	120	184
	174	150	192
	180	130	120
	117	130	142
Mice bearing spontaneous tumors	190	171	150
	210	141	230
	174	185	225
	185	171	148
	192	140	190
	165	173	196

made just before, and at the stated periods after, the subcutaneous injection of 0.125 cc. of this extract. In a similar manner extracts of spontaneous mouse tumors were prepared, and as with the spleen animals, each extract was injected into the animal from which the tissue was derived. In a third group of animals bearing transplanted Jensen rat sarcomata twenty-one days old, the tumors were removed, emulsified, and extracted,

as were the other tissues, the extract in turn being injected into the animal from which it was derived. The data of this experiment are presented in table 3.

TABLE 3

Effect of injections of autologous protein extracts. All figures indicate milligrams of sugar per 100 cc.

	ZERO HOUR	45 MINUTES	120 MINUTES
Normal rats injected with autologous spleen extract	170	172	172
	180	178	180
	168	165	164
	148	150	146
	160	159	157
	152	154	152
	148	148	150
	190	192	194
	165	161	160
	158	159	155
Normal rats injected with autologous Jensen sarcoma extract	150	152	150
	160	160	158
	220	140	142
	146	148	150
	195	124	210
	134	140	136
	170	140	195
	161	160	163
	157	157	158
	165	121	210
Normal mice injected with autologous spontaneous tumor extract	180	180	178
	164	166	170
	176	176	178
	200	200	200
	184	183	184
	190	192	188

Note that autologous protein extracts induce no reaction in normal animals or in spontaneous tumor-bearing animals; but that autologous transplanted tumors sometimes give rise to a reaction.

As is shown in that table, autologous spleen extract and autologous spontaneous tumor extract did not give rise to a reaction, or to put the matter more exactly, gave rise to a reaction so small

(variations of 10 mgm. or less have arbitrarily been classed as no reaction) as to be within the factor of error of the method of determination. In contrast to this, extracts of autologous transplanted tumor sometimes gave rise to a reaction, and sometimes did not. While, broadly speaking, a transplanted tumor may be considered autologous, in a strict sense it is not, since it arises from a group of cells which several years ago were autologous to another animal. The present host merely furnishes a residence and nourishment, so to speak.

TABLE 4

Animals bearing transplanted tumors injected with autologous tumor extract. Sugar estimation made just before injection and again one hour after. Variations of 10 milligrams or less classed as negative glycemic reaction

TUMOR STRAIN	NEGATIVE REACTION				POSITIVE REACTION			
	Receding		Growing		Receding		Growing	
Flexner	0		18 (75%)		0		6 (25%)	
Jensen	6 (28%)		1 (4%)		13 (59%)		2 (9%)	
Mouse 11	1 (13%)		7 (87%)		0		0	
	Average figures: Blood sugar							
Flexner			438	490			461	467
Jensen	480	480	462	464	492	444	454	490
Mouse 11	444	456	512	515				

Summary. Of those animals, tumor strain not separated, which showed receding tumors, 65 per cent gave a glycemic reaction; while of those which showed growing tumors, 76 per cent gave no glycemic reaction.

A further investigation of the variations occurring in animals bearing transplanted tumors was undertaken. A number of animals were inoculated with the usual dose of either the Flexner-Jobling rat carcinoma, the Jensen rat sarcoma, or Crocker Fund mouse carcinoma no. 11. After twenty-one days of tumor growth, portions of the tumor were removed, and extracted in distilled water; and the extract, in dosage of 0.125 cc., was injected subcutaneously into the animal from which the tumor had been removed. Blood sugar estimations were made at the same time periods and according to the same method as in the previous experiments.

The summarized data are presented in table 4. Of those animals, tumor strain not separated, which showed receding tumors, 65 per cent gave a glycemic reaction, i.e., showed variations of more than 10 mgm. in the three estimations made. In contrast to these figures, but 24 per cent of the animals in which the tumor continued to grow gave a glycemic reaction.

The experiments so far recorded have shown that heterologous protein extracts are capable of inducing a hyperglycemia in normal as well as in spontaneous and transplanted tumor-bearing animals. On the other hand, autologous protein extracts do not induce a hyperglycemia in normal or in spontaneous tumor-bearing animals, though occasionally they may in animals bearing transplanted tumors.

In a final experiment, the effect of injections of homologous protein in normal animals, in transplanted tumor-bearing animals, and in spontaneous tumor-bearing animals was studied. In this experiment several Flexner-Jobling rat carcinomata, twenty-one days old, were emulsified and extracted with distilled water. In a similar manner extracts of Jensen rat sarcoma and of rat spleen were prepared. These extracts were injected in a dosage of 0.125 cc. into animals bearing the Flexner tumor, into those bearing the Jensen tumor, and into normal animals. Extracts of mouse carcinoma 11 and of mouse spleen were also prepared and injected in a dosage of 0.125 cc. into animals bearing spontaneous tumors. Blood sugar estimations were made as before at the stated time periods. The data which are given in tables 5 and 6 show a rather surprising result, in that homologous protein as exemplified by the extracts of either the Jensen or Flexner tumor or of rat spleen did induce hyperglycemia in normal rats, and in rats bearing either one of the two transplantable tumor strains, while animals bearing spontaneous malignant tumors in a large percentage failed to give hyperglycemia when injected with either homologous tumor or normal tissue.

Speculations based upon the results of the experiments recorded here are interesting. It is apparent that there is a biological difference between a majority of animals bearing spon-

taneous tumors and other animals, which difference is demonstrable by the failure of spontaneous malignant tumor-bearing animals to respond by hyperglycemia upon the injection of

TABLE 5

Effect of injections of homologous protein extracts. All figures indicate milligrams of sugar per 100 cc.

	ZERO HOUR	45 MINUTES	120 MINUTES
Normal rats injected with Flexner tumor extract..	141	178	130
	139	152	192
	156	194	130
	171	162	141
Normal rats injected with Jensen tumor extract..	132	155	175
	148	185	196
	151	132	190
	146	121	184
Normal rats injected with spleen (rat) extract....	155	171	135
	151	162	185
	157	174	196
	160	130	110
Rats bearing Flexner tumor injected with spleen (rat) extract....................................	144	160	130
	164	150	100
	176	168	150
	190	160	130
Rats bearing Flexner tumor injected with Flexner tumor extract................................	148	124	162
	162	120	140
	156	160	132
	160	118	124
Rats bearing Jensen tumor injected with Flexner tumor extract................................	170	160	110
	148	175	195
	161	195	130
	136	152	183

homologous protein extract. That some biological difference exists between normal and tumor-bearing individuals is suggested in many other ways; for example, by the clinical observation that an irritant known to produce carcinoma in some indi-

TABLE 6

Glycemic reaction in animals bearing spontaneous tumors when injected with homologous protein extract (mouse spleen)

ANIMAL NUMBER	DIAGNOSIS	PREOPERATIVE	
		Zero hour	1 hour
83	Sarcoma of neck	320	328
84	Fibroma of breast	304	336
85	Adenoma of breast	440	404
1741	Carcinoma of breast	160	160
1746	Carcinoma of breast	150	148
1752	Carcinoma of breast	158	156
1755	Carcinoma of breast	176	178
1760	Carcinoma of breast	540	472
1761	Carcinoma of breast	460	520
1762	Carcinoma of breast	528	524
1763	Carcinoma of breast	504	504
1764	Carcinoma of breast	520	524
1765	Carcinoma of breast	416	460
1766	Carcinoma of breast	448	460
1767	Carcinoma of breast	440	504
1771	Carcinoma of breast	452	520
1772	Carcinoma of breast	408	408
1773	Carcinoma of breast	444	448
1774	Carcinoma of breast	476	476
1777	Carcinoma of breast	508	508
1778	Carcinoma of breast	452	448
1779	Carcinoma of breast	368	436
1781	Carcinoma of breast	540	540
1782	Carcinoma of breast	536	568
1783	Carcinoma of breast	500	500
1784	Carcinoma of breast	564	564
1787	Carcinoma of breast	524	524
1791	Carcinoma of breast	536	524
1792	Carcinoma of breast	532	532
1793	Carcinoma of breast	560	560
1794	Carcinoma of breast	545	548

Of 28 animals bearing spontaneous tumors, 75 per cent gave no glycemic reaction when injected with homologous protein. In the later two-thirds of this series 0.05 cc. of blood was taken for the sugar estimation instead of 0.2 cc. as in the previous experiments. It has been our experience that when such small amounts are used the values obtained are much higher than when larger amounts of blood are used.

viduals does not produce it in all. If the irritant were the sole factor, a neoplasm should follow in the greater proportion of all exposed to the same irritant. How long this biological difference exists in spontaneous malignant tumor-bearing animals before the tumor develops can only be answered after further investigations.

Another speculation concerns the etiological factors which may bring about a condition in which the individual does not react after the injection of homologous protein. The internal secretions are to be seriously considered as etiological factors in producing this biological change.

CONCLUSIONS

A transient hyperglycemia occurs in rats after injection of adrenalin. The type of this hyperglycemia is not distinctive nor characteristic after inoculation with transplantable tumor, irrespective as to whether or not the tumor graft grows.

Heterologous protein extracts induce hyperglycemia in normal animals and in those bearing transplantable tumors, as well as spontaneous tumor-bearing animals.

Autologous protein extracts do not cause a hyperglycemia in normal or spontaneous tumor-bearing animals; they occasionally cause a hyperglycemia in animals bearing transplantable tumors, if the autologous tissue be the tumor itself.

Homologous protein extracts cause a hyperglycemia in normal animals and in animals bearing transplantable malignant tumors; they do not cause a hyperglycemia in animals in which spontaneous malignant tumors are growing.

Homologous protein, which is able to induce immunity against subsequent inoculation of a tumor, gives rise to a hyperglycemia, while autologous protein extract, which has not the power of conferring immunity, does not give rise to a hyperglycemia when injected into normal animals. A negative glycemic reaction, therefore, might be interpreted as an inability of the organism to produce antibodies against the substance injected. That such an interpretation is probably correct is

shown by the fact that of animals bearing transplanted tumors, 75 per cent of those in which the tumor grows fail to give a glycemic reaction when injected with extracts of protein of the tumor, while those animals in which the tumor recedes give a glycemic reaction in 69 per cent of the cases. Animals in which the tumor recedes probably produce antibodies against the tumor, even though these cannot be demonstrated by any method at present known.

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SKIN INVOLVEMENT IN BREAST CANCER WITH REFERENCE TO ITS BEARING ON THE INTERPRETATION OF APPEARANCES OF TRANSITION BETWEEN NORMAL EPITHELIUM AND CANCER[1]

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Whether carcinoma ever extends by the transformation of normal epithelial cells into cancer cells at the advancing tumor margin has been a much debated question. The principal evidence in favor of spread in this manner is found in the histological pictures showing all stages of apparent gradual transformation of normal epithelial cells into malignant cells, seen notably in epitheliomata and in cancers of the intestine and duct cancers of the breast. If these are true pictures, their bearing upon the nature of cancer is of the first importance, indicating that the properties of malignancy may be conferred upon normal cells by an influence outside themselves, and that tumors need not necessarily grow exclusively from their own cells.

Borrmann (1), writing of epithelioma and arguing for the origin of tumors from foci of misplaced embryonic tissue, considers that the apparent transitions are to be explained as secondary union of the cancer with the normal epithelium. Ribbert (2), in a discussion of Paget's disease of the nipple, has expressed the same opinion: "Cancer here (of the breast) as elsewhere and like all other tumors, after it is once fully established, grows only from itself. This is a fundamental consideration. . . ."

Janeway (3) has summarized and endorsed the opposite view, that the pictures observed are true transformations. Writing of

[1] This work was done during the tenure of a fellowship of the China Medical Board, Rockefeller Foundation.

epithelioma, he says, "The new growth increases in size by a transforming influence upon the adjacent healthy epithelial cells with which it is in direct connection." More recently Ewing (4) has definitely expressed this view. In the breast, for instance, "some acinar and many duct carcinomas arising at one focus, gradually extend over adjoining areas by a gradual transformation of duct and acinar epithelium into neoplastic cells." A similar conception of the spread of carcinoma of the large intestine is mentioned (5). The question has not, therefore, been answered with finality and any evidence bearing on it is worthy of record.

The researches above referred to have been made by histological study of the relation of tumors to surrounding normal epithelium in which they have arisen. The tumor cells, especially in epitheliomata, still bear a more or less close resemblance to the surrounding normal cells and are of necessity in contact with them (unless the normal epithelium has been destroyed well in advance of the cancer), so that every opportunity is offered for the production of deceptive appearances of transition. It is reasonable to expect some light to be thrown on the question by a study of contact between malignant and non-malignant epithelia of different histological type. The problem has been approached experimentally from this point of view by Rous (6) who demonstrated that a transplantable adenocarcinoma (the Flexner-Jobling tumor) would unite directly with normal regenerating skin epithelium of a granulating wound to produce histological pictures of simple union and of *apparent* gradual transition, without evidence which could be interpreted as showing that the skin cells were actually changed into tumor cells. Previous work with simultaneous grafting of embryo tissues and tumor (7) had shown that the cells of an adenocarcinoma may unite secondarily with normal epithelial cells of quite different histological type and intermingle with them.

We are approaching the problem on experimental animals from a somewhat different point of view. But in the meantime it has seemed worth while to study the experiment, carried out by nature in the human subject, of allowing cancer to grow into contact with normal epithelium, which may be observed when-

ever cancer of the breast involves the overlying skin. The conditions are ideal, inasmuch as the tumor is one arising from the individual's own cells rather than from transplanted material, and, furthermore, the mammary epithelium from which the tumor arises is intimately related embryologically with the skin epithelium, while it is yet sufficiently different in histological appearance to be readily differentiated.

The object of this paper, therefore, is to report a study of the histological pictures seen when skin epithelium has been "exposed" to approaching mammary carcinoma and to show that in the cases studied, no pictures of transformation occurred, but that appearances of histological union were seen which, except for the well-marked difference in morphology between the cells of the two types of epithelium, might be mistaken for true transformations.

The processes which go on when deep carcinoma of the breast approaches the skin have been summarized by Ribbert (2): "When a cancer approaches the epidermis either it destroys it as a whole by compression, or its alveoli press on the basal layer, blend with it and break through it here and there, or they grow inside the epidermis, building in it epithelial nests and columns." Each of these processes has been observed in the material studied for this paper. The variation in the general relation of advancing carcinoma to overlying skin has been striking. In some instances a single column of cancer cells has approached and penetrated the entire thickness of the epidermis well in advance of the main tumor mass (fig. 1). In a number of cases, especially at the margins of ulcers, islands of cancer cells have been found immediately against the skin epithelium without the interposition of basement membrane. The arrangement of the groups of cancer and of epithelial cells, however, has not been such as to suggest a true union but only a very close apposition (fig. 2).

In another type of approach a large area of cancer has been circumscribed against the epidermis with a narrow margin of connective tissue intervening (fig. 3). In still other cases, extraordinary pictures of hypertrophy of the epithelium downward in fine processes interlacing with equally fine strands of cancer have presented themselves (fig. 4).

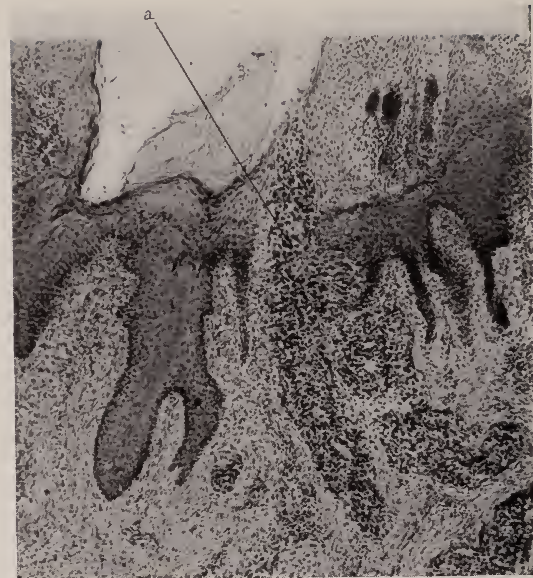

FIG. 1. PATH. NO. 23931
a, A single column of cancer cells penetrating the epidermis

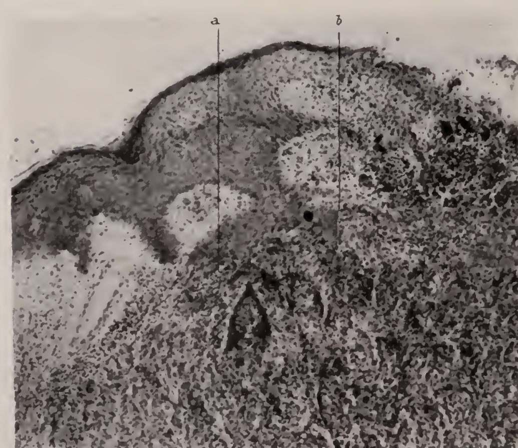

FIG. 2. PATH. NO. 25111
At an ulcer margin. *a*, An island of cancer cells in close apposition to skin epithelium which surrounds it on three sides. No basement membrane is seen, but there is no interdigitation of the two types of cells, the arrangement indicating only close apposition and not secondary union. *b*, Point of contact of another island of cancer with skin epithelium; basement membrane not yet destroyed.

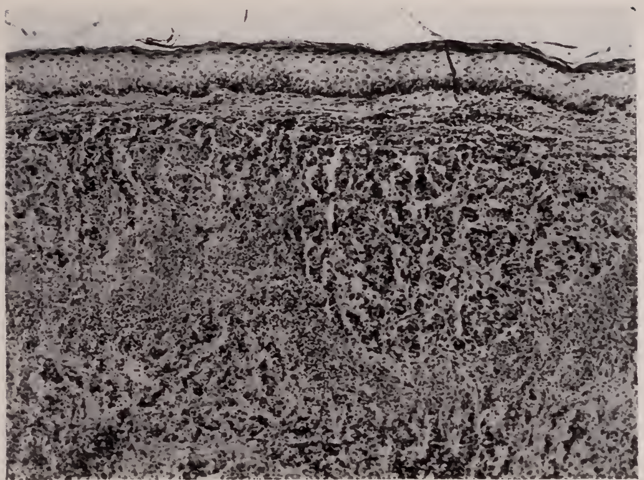

Fig. 3. Path. No. 23730

A second type of approach of carcinoma to skin. Broad area of cancer circumscribed against flattened epithelium with narrow zone of intervening connective tissue. No hyperplasia of skin epithelium. No secondary union.

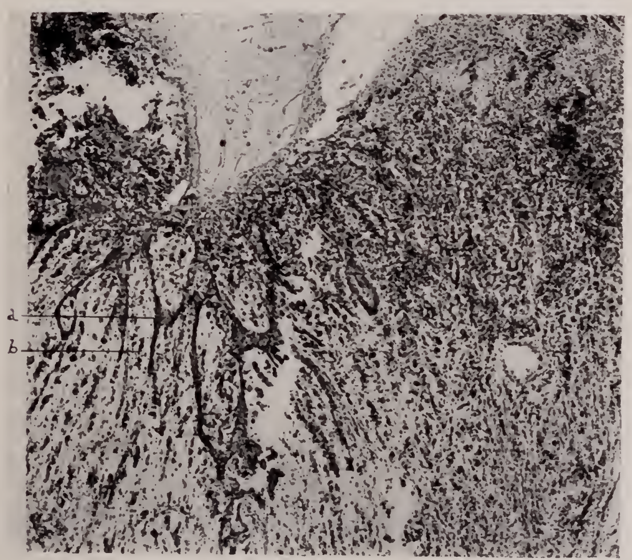

Fig. 4. Path. No. 14251

Third type of approach. Marked hyperplasia of skin epithelium in thin processes interlacing with fine strands of cancer. *a,* Process of skin epithelium. *b,* Strand of cancer.

It is in the last-mentioned type of picture that the main interest of the study has centered, for in the relation of the fine strands of cancer and the fine hypertrophic processes of skin epithelium have been found the appearances of secondary union, the interpretation of which is in question. Figure 5 shows a process of downgrowing skin epithelium which has preserved its basement membrane against the cancer strands at its side, but shows

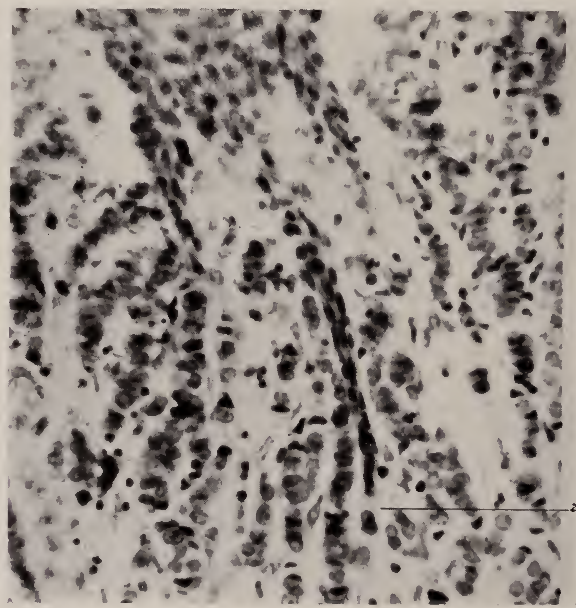

Fig. 5. Path. No. 14251

a, Cancer cells in apparent secondary union with a narrow process of epithelium at its tip.

apparent union with the cancer cells at its tip. The connection between the two, evident under the microscope, is shown but faintly in the photomicrograph, and for this reason the picture is perhaps not as convincing as that shown in figure 6, from another part of the same specimen, in which a strand of cancer cells lies parallel to, and in intimate contact with, a process of epithelium and the highest cancer cell has definitely interdigitated with the skin cells. The change in its shape is obviously due to

this interdigitation, and it cannot be regarded as a transitional form between normal and cancer cells. The non-malignant skin epithelium is readily distinguished by its fusiform or oval, small, deep-staining nuclei, its spindle-shaped cells with rather dark-staining protoplasm, arranged in a more or less orderly manner in the processes; the cancer cells by their large, irregular, more vesicular nuclei, their scant, ill-defined, and lightly-stained protoplasm, and the irregularity of their arrangement in strands and small islets. There are no intermediate types to be seen. If, however, the carcinoma were of the prickle-cell variety, confusion might easily arise in attempting to designate the last malignant and the first non-malignant cell.

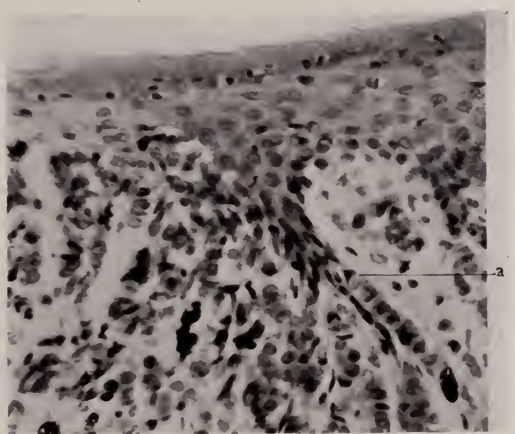

Fig. 6. Path. No. 14251

a, A cell at the end of a cancer strand interdigitating in apparent secondary union with cells of an epithelial process.

In figure 7 a cell belonging to a small island of cancer has interdigitated with the cells of a process of skin epithelium, approaching in this instance from the side. In spite of the apparent actual histological union it is possible, on account of the marked difference in morphology between the two types of cells, to designate with certainty the last mammary cancer cell and the first epidermal cells, and again no intermediate forms are to be seen.

The material on which this study has been made consists of 475 specimens of cancer of the breast of fully developed scirrhous or medullary type which have been received in the Surgical Pathology Laboratory of the Johns Hopkins Hospital during the past ten years. Of this number 74 showed histological involvement, either of the nipple or of the skin overlying the breast. In 24 of these, however, the cancer had reached only to the lower layers of the derma and showed close approach only to the hair

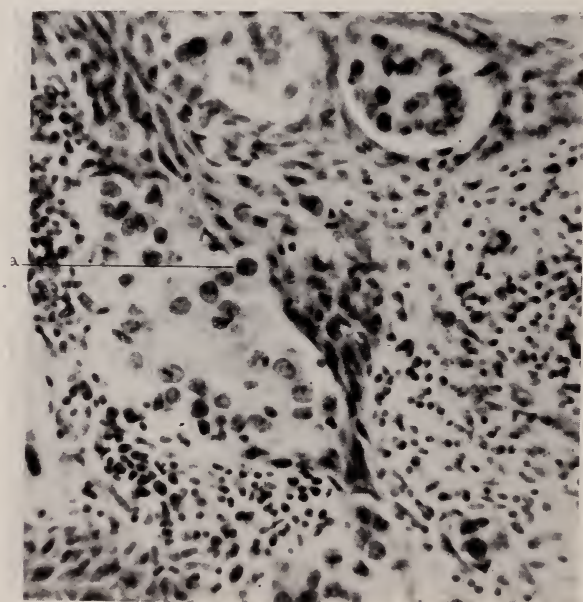

Fig. 7. Path. No. 12215

a, A cell from a cancer island interdigitating with cells of a process of skin epithelium. Approach from the side of the process.

follicles or skin glands—incidentally without showing any appearances of histological union with these structures. It has been considered, therefore, that true "exposure" of the epidermal epithelium to carcinoma has occurred 50 times in the 475 breast cancers observed. Of these 50 exposures 16 showed intimate approximation of cancer to skin cells, but in only 7 of these were seen pictures of apparent histological union such as is illustrated

in figures 5, 6, and 7. In none of these 7 cases of secondary union was seen any suggestion of transformation of non-malignant into malignant cells. Study of serial sections in a number of specimens has not changed the interpretation of the appearances at any given point.

It is of interest that all the pictures of apparent secondary union found in these cases occurred only where the skin epithelium was definitely hyperplastic, i.e., in those cases in which the skin over the cancer showed the type of reaction illustrated in figure 4. In the experimental work mentioned above, Rous also obtained secondary union between tumor cells and epithelium in hyperplastic activity, either regenerating epithelium on a granulating wound (6) or transplanted embryonic epithelium (7). Active growth of both of two tissues is therefore favorable, if not essential, for secondary union. If the uniting epithelia are dissimilar the cells of each retain their identifying characteristics.

These considerations suggest a reasonable explanation of the apparently perfect transition pictures seen between cancer and surrounding normal epithelium. Certainly hyperplasia of non-malignant epithelium is commonly seen at the margins of cancer, the effect of which is to alter the general morphology of the normal cells in many respects toward that of the tumor cells.[2] Cancer cells, on the other hand, vary from marked distortion to forms nearly, if not quite, normal in morphology. Gradual transition may easily be simulated by this variation of each type toward the other, the line of demarcation between cancer and normal epithelium being obscured by their secondary union.

SUMMARY

It is not the purpose of this paper to assert that cancer never extends by transformation at the periphery of normal epithelial into malignant cells, but an attempt has been made to show that, in a considerable number of cases in which conditions for

[2] This reaction of normal epithelium is by no means specific for cancer. Exactly similar hyperplasia is characteristically observed in epithelium at the margins of benign epitheliomata and of ulcers due to tuberculosis, syphilis, or chronic inflammation. Vide, Councilman: Bull. Johns Hopkins Hosp. 1890, No. 2.

the occurrence of this transformation were theoretically very favorable, it has not been observed. Conclusions may be summarized as follows:

1. In 50 instances of "exposure" of epidermal epithelium to cancer approaching from below seen in 475 mammary carcinomas, no picture was observed which could be interpreted as transformation of normal into malignant cells.

2. In 7 cases, however, secondary histological union apparently occurred, which, but for the distinctive morphological appearance of the cancer and the skin epithelial cells, might be mistaken for transformation.

3. In so far as this evidence weighs, it is against the transformation of normal epithelial cells into cancer at the advancing margin of tumor.

4. The pictures of secondary union observed in these cases have occurred only in the presence of hyperplasia of the normal epithelium, and with this in mind a reasonable explanation is suggested of the apparent transitions seen at the margins of tumors, the cells of which are more nearly like those of the surrounding epithelium.

I am indebted to Dr. Joseph C. Bloodgood for permission to use the material in this laboratory and for criticism and suggestions; and to Mr. Herman Schapiro for the accompanying photomicrographs.

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THE RESPONSE OF THE ANIMAL ORGANISM TO REPEATED INJECTIONS OF AN ACTIVE DEPOSIT OF RADIUM EMANATION

INTRAVENOUS INJECTIONS IN DOGS

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Following intravenous or subcutaneous injections of an "active deposit" of radium emanation, the writer found (1) in the case of white rats, that pathological changes had resulted in the liver, kidneys, spleen, bone marrow, etc., of these animals; and since the methods employed in these experiments were being used in the treatment of certain types of human cancer, it was decided to run a parallel set of experiments on a larger animal, the dog, and therein to determine the severity of the physiological reactions. To this end a complete urine analysis was made each day, before and after the treatments; frequent blood counts and differentials were made; the temperature and weight reactions were recorded, and finally a histological study was made of the organs removed at autopsy.

This experiment was undertaken in collaboration with Miss Ruth Theis of the chemical department of the Memorial Hospital. We will report elsewhere on the detailed findings of the urine analysis, and mention of them will be made in this paper only in so far as they aid in the interpretation of what might be called the general clinical aspects of the problem (2).

APPARATUS AND METHODS

Two female dogs were the subjects of the experiments. Dog I was a Dalmatian and weighed $32\frac{1}{2}$ pounds when first obtained

by the laboratory. Dog II was a mongrel, with the bull dog type predominating, and weighed nearly 28 pounds at the beginning of the experiment.

While the dog was being treated it was catheterized at a definite time each day. It was kept in a metal metabolism cage, in which the urine and feces were collected separately. The catheterized urine completed the twenty-four hour specimen that was used as a basis of comparison of the urine analysis. Although the dogs were under observation for a comparatively long time, eighty days in the case of dog I, no bladder infections were encountered. Each dog was trained to lie quietly on its back in an ordinary trough shaped animal board, its legs were loosely tied, and catheterization was effected by means of a silver catheter. These arrangements made it possible for one person to do the work unaided by an assistant. The bladder was emptied and then washed two or three times with sterile warm water, which was added to the day's collection of urine, together with the washings from the floor of the metabolism cage. After the washing with sterile water, the bladder was again flushed with a saturated solution of boric acid. Immediately after this operation the dog was fed its daily amount of food previously prepared and measured according to a definite formula.

The "active deposit" of radium emanation was prepared as described in the previous article (1). It consisted of a solution in which radium emanation had been previously deposited upon common salt, which subsequently was dissolved in sufficient sterile water to bring the liquid to the strength of a physiological salt solution. This solution contained all the properties of radium metal itself.

The intravenous injections were effected as follows: First the dog was placed on its back on the animal board, and held firmly in position. Then its ear, previously shaved, was warmed by several applications of hot cloths until the ear veins became prominent. The ear was washed with alcohol, and by means of a fine needle and a 2 cc. Luer syringe, the activated solution was slowly allowed to enter the general circulation. About 2 cc. of solution were injected at each treatment. The syringe was

covered by a lead shield which acted as a protection for the fingers of the operator.

The blood samples were also obtained from the ear. Rectal temperatures were taken each day, and at more frequent intervals during the period of treatment. The dog was weighed every day during the time when significant weight changes were occurring.

Throughout the experiment an injection of the active deposit was not repeated until the examination of the urine showed that the metabolism of the animal had recovered from the previous treatment.

A. EXPERIMENTAL RESULTS FOR DOG I

First treatment. In the case of dog I the treatments were started at the beginning of March and continued until the last week in the following May. During this period the animal received four intravenous injections of the radioactive solution, the total amount being 231.8 mc. The first treatment consisted of an injection of 95.3 mc. This was followed by a period during which the animal was inactive. The feces were semifluid and food was eaten only after much persuasion. This condition lasted for only two days, for on the morning of the third, the dog's appetite had completely recovered, and the feces were again normal. The second treatment was not given until a month later, and during that time the animal appeared normal, except for a temporary inexplicable loss of appetite on the thirteenth day of the experiment, and on the twenty-third and twenty-fourth days.

Figure 1 shows that following the first treatment there was a considerable drop in the number of white blood-cells, which reached their lowest point on the eleventh day of the experiment. The number of cells before treatment was 10,250 and ten days later their total was only 4200, but from then on there was a steady recovery until the normal number was again reached just before the second treatment. Immediately after the injection, the number of red blood-cells remained practically unaltered, and, in fact, while the white cells were rapidly decreasing the reds held their own and slightly increased in number.

As indicated in table 1, on the eleventh day of the experiment the differential showed that an interesting change had taken place in the composition of the white blood-cells. The percentage of polynuclear leucocytes had gradually been reduced from 83 to 64, and the relative percentage of lymphocytes correspondingly increased from 16 to 29, while the eosinophiles went from 1

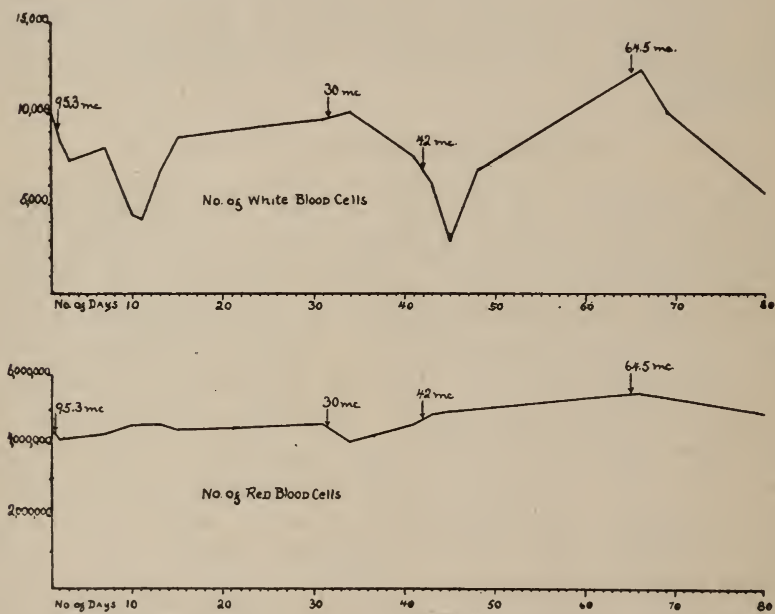

Fig. 1. Record of Red and White Blood-Cell Changes in Dog I Following Repeated Intravenous Injections of the Active Deposit of Radium Emanation

The arrows indicate the time when the injections were given, and immediately above them is indicated the dosage, expressed in millicuries.

to 6 per cent, and 1 per cent of myelocytes was found. There was also a slight decrease in hemoglobin. Before the next treatment the differential was again normal.

The examination of the urine showed that in response to the injection there was an increase in the total nitrogen and urea, and a considerable increase in the uric acid and phosphates, but,

as in the case of the blood changes, the metabolism was also back to normal before the next treatment.

Second treatment. The second injection occurred on the thirty-first day of the experiment. A much smaller dose was given,

TABLE 1

Complete record of the blood counts for dog I

RADIUM TREATMENTS	NUMBER OF DAYS	NUMBER OF RED CELLS	NUMBER OF WHITE CELLS	PERCENTAGE HAEMOGLOBIN	PERCENTAGE POLYNUCLEAR LEUCOCYTES	PERCENTAGE LYMPHOCYTES	PERCENTAGE EOSINOPHILES	PERCENTAGE MYELOCYTES
	1	4,400,000	10,250	80	83	16	1	0
First radium treatment, 95.3 mc.	1							
	2	4,112,000	8,300	80				
	3	4,256,000	7,450	70	82	18	0	0
	7	4,336,000	8,200	85				
	10	4,600,000	4,400	85	70	28	2	0
	11		4,200	75	64	29	6	1
	13	4,640,000	6,900	75	77	20	3	0
	15	4,440,000	8,900	75	85	11	4	0
	22	4,500,000	9,300	75				
	31	4,664,000	9,900	80	83	15	2	0
Second radium treatment, 30 mc.	31							
	34	4,136,000	10,200	80	86	14	0	0
	41	4,690,000	7,600	80				
Third radium treatment, 42 mc.	42							
	43	4,890,000	6,150	80				
	45	4,976,000	2,900	80				
	48		6,950					
Fourth radium treatment, 64.5 mc.	65							
	66	5,544,000	12,500	75	81	17	2	0
	69	5,400,000	10,100	80	76	17	7	0
	80	4,980,000	5,600	80	75	25	0	0

consisting of 30 mc., and save for the fact that the feces were slightly more fluid than normal, the dog remained well and active. As shown in figure 2, the temperature during this period showed considerable irregularity, with generally low temperatures for a few days.

Three days after the treatment the white blood-cells were slightly increased in number, but from that point until the third treatment the cells were decreased about 25 per cent. The red blood-cells were slightly lowered, but regained their normal number before the next treatment. No significant change was noted in the differential.

There was a slight rise in the total nitrogen, the urea, and the total phosphates of the urine, while the uric acid remained at about the same level.

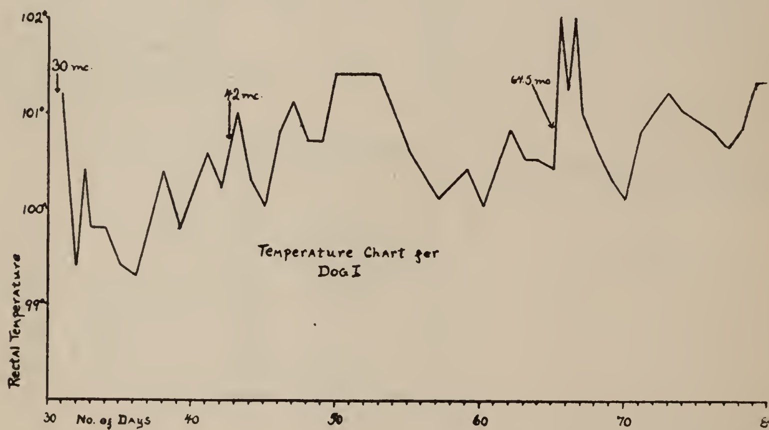

Fig. 2. Temperature Chart for Dog I

The arrows indicate the time when the injections were given. The record was not begun until after the second treatment.

Third treatment. A third injection of 42 mc. was given on the forty-second day of the experiment. It was followed by a slight amount of diarrhea, loss of appetite which lasted for ten days, and a decrease in weight amounting to two pounds. There was also a sharp rise in temperature, with irregular and generally high temperatures for several days, during which the feces were hard and dry. There was a further reduction in the number of white blood-cells to 2900, which number was recorded three days after the injection. From this point the white cells rapidly increased, until at the time of the next treatment they were 12,500 in number.

The hemoglobin remained at 80 per cent. The number of red blood-cells increased, so that before the animal was again injected there were approximately a million more red cells present than there were at the beginning of the experiment. Just after the third treatment it was noted that the blood tended to clot with unusual rapidity.

The examination of the urine showed a considerable increase in the total nitrogen, urea, creatinine, uric acid, and the total phosphates.

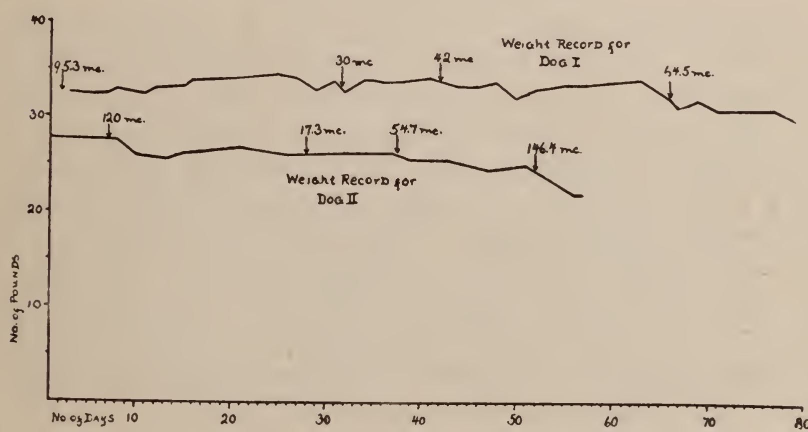

FIG. 3. RECORD OF WEIGHT CHANGES FOR DOG I AND DOG II FOLLOWING REPEATED INTRAVENOUS INJECTIONS OF THE ACTIVE DEPOSIT OF RADIUM EMANATION

The arrows indicate the time when the injections were given

Fourth treatment. Sixty-four and a half millicuries were injected on the sixty-fifth day of the experiment. This treatment was followed by severe vomiting, constipation, the excretion of a small amount of solid, dry feces, and a sudden rise in temperature to 102°. At the end of the following day the temperature again reached the same high level before finally receding. The animal refused to eat for two days, and as indicated in figure 3, there was a steady loss of weight amounting to $3\frac{1}{4}$ pounds. The animal was inactive and obviously seriously ill.

The white blood-cells were reduced in number to 5600, but the red blood-cells were only slightly decreased. The differential showed a relative increase in the number of lymphocytes at the expense of the polynuclear leucocytes. The fact that the animal refused to eat after the fourth treatment made the chemical analysis of the urine unreliable. There was, however, a decided increase in the amount of urine excreted each day.

At this time the dog was killed by means of ether.

Histological report for dog I

The histological study of the organs showed an intensely congested liver, with some capillary varicosities and severe granular degeneration of most of the parenchyma cells. The kidneys were also intensely congested, showing a moderate amount of degeneration of the tubule cells. The malpighian bodies of the spleen were prominent; the organ was congested, and the pulp considerably drained of cells. There was marked congestion of the colon associated with active mucus production. The lymph-nodes showed extreme congestion, especially of the pulp. The lymph-follicles were reduced in size, but the reticulum cells were generally increased in number. No definite changes were noted in the lungs, stomach, small intestine, or thyroid.

B. EXPERIMENTAL RESULTS FOR DOG II

First treatment. The experimental work with dog II began in the first part of June and continued until the end of July. Four injections were given, the total amount of radium emanation amounting to 338.4 mc. The initial treatment consisted of 120 mc. injected on the seventh day of the experiment. As indicated in figure 4, this was followed by irregularly lowered temperature for several days following the injection. No gastric symptoms were noted until three days after treatment, when vomiting occurred; the animal became dull and inactive, and refused most of its food. Figure 3 shows there was a loss in weight amounting to 2 pounds, occurring within six days. As shown in figure 5 there was a marked decrease in the number of white blood-cells;

from 14,400 before treatment to 2150 seven days later. There was a temporary fall in the hemoglobin from 85 to 75 per cent, accompanying a decrease of about two million red cells. As shown in table 2, the differential remained normal, except for a slight relative increase of lymphocytes. The examination of the urine showed a large increase in uric acid and the total phosphates.

Second treatment. A small dose, consisting of 17.3 mc., was injected on the twenty-eighth day of the experiment. After this treatment there was at first a temporary drop in the body tem-

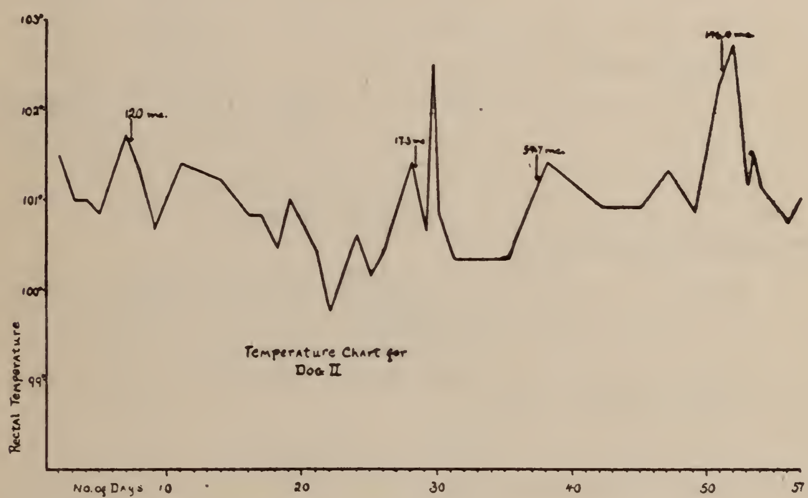

Fig. 4. Temperature Chart for Dog II

The arrows indicate the time when the injections were given

perature, followed by a rapid increase at the end of the following day, at which time 102.5° was reached. No other change was noted in the animal's general condition. It remained active, and ate with its usual good appetite. There was a steady decrease in the number of white blood-cells, the hemoglobin remained normal, but the red blood-cells continued to increase slowly. The weight remained stationary, as indicated in figure 3. The analysis of the urine showed only a slight increase in the total nitrogen, the creatinine, the uric acid, and the total phosphates.

Third treatment. On the thirty-seventh day of the experiment the animal received an injection of 54.7 mc. Following this there was a slight temporary increase in temperature, during which time the animal was somewhat inactive for three successive days, but showed no signs of digestive disturbances, except for a temporary refusal of food on the fourth day. During this

TABLE 2

Complete record of the blood counts for dog II

RADIUM TREATMENTS	NUMBER OF DAYS	NUMBER OF RED CELLS	NUMBER OF WHITE CELLS	PERCENTAGE HAEMOGLOBIN	PERCENTAGE POLYNUCLEAR LEUCOCYTES	PERCENTAGE LYMPHOCYTES	PERCENTAGE EOSINOPHILES	PERCENTAGE MYELOCYTES	PERCENTAGE NORMOBLASTS
	3	6,400,000	13,800	85	79	19	1	0	1
	4	5,924,000	14,400	85					
First radium treatment, 120 mc.	7								
	8	5,600,000	8,900	85	82	17	1	0	0
	9	5,280,000	4,550	80	79	18	3	0	0
	11	4,880,000	2,815	75	84	16	0	0	0
	14	4,680,000	2,150	80					
	28	4,976,000	5,700	85	75	23	2	0	0
Second radium treatment, 17.3 mc.	28								
	29	5,250,000	4,450	85					
Third radium treatment, 54.7 mc.	37								
	38	5,800,000	3,650	85	83	14	3	0	0
	49	5,814,000	4,100	80	85	12	3	0	0
Fourth radium treatment, 146.4 mc.	52								
	53	5,200,000	2,600	85	95	3	2	0	0
	57	4,520,000	1,400	85					

period the animal lost $1\frac{1}{2}$ pounds in weight. The number of red and white blood-cells remained fairly constant, and yet the differential showed a relative decrease in the percentage of lymphocytes, with a corresponding relative increase in the polynuclear leucocytes and the formation of a few eosinophilic cells. The urine showed an increase in the total nitrogen and the phosphates, while the creatinine stayed at about the same level.

Fourth treatment. The final injection was a large one, and consisted of 146.4 mc. administered on the fifty-second day of the experiment. Just before the treatment the temperature went as high as 102.3°, and after the treatment it reached 102.7°

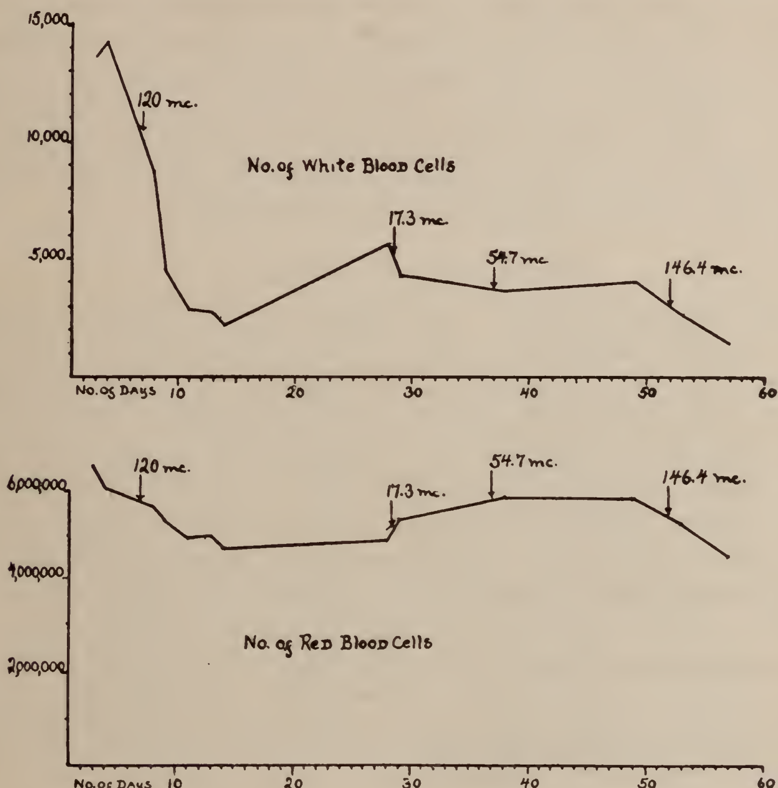

Fig. 5. Record of Red and White Blood-Cell Changes in Dog II Following Repeated Intravenous Injections of the Active Deposit of Radium Emanation

The arrows indicate the time when the injections were given

and then as suddenly receded. A few hours after the treatment a considerable amount of mucus covered vomitus was found in the cage, as well as a large quantity of fecal matter, semi-solid and odorless, and also covered with mucus. The animal refused to eat during the rest of the experiment except for an occa-

sional small amount of food. It lost 2 pounds in weight within five days, and remained seriously ill and inactive. The white blood-cells decreased still further in number until at the end of the experiment they were as low as 1400 cells. The differential showed a very decided reduction in the percentage of lymphocytes. These cells were reduced to 3 per cent, while a corresponding relative increase in the polynuclear leucocytes was recorded, amounting to 95 per cent. The hemoglobin remained normal and the red blood-cells were but slightly decreased in number. The urine analysis was unreliable, because the animal refused to eat. The animal was killed by ether anesthesia.

Histological report for dog II

The histological study of the organs showed a general fatty and granular degeneration of the liver, associated with capillary congestion. The kidneys showed a slight granular degeneration of the tubule cells, with general venous congestion. There was a marked old thickening of the trabeculae of the spleen, a heavy pigmentation of the parenchyma cells, associated with small and scanty follicles. The splenic pulp showed considerable congestion and fibrosis. The bone marrow from the head of the femur was devoid of lymphoid cells, which were largely replaced by fat. The lungs were congested, and showed a slight degree of emphysema. There was a small amount of catarrhal exfoliation of the sinus cells of the lymph-nodes. The pancreas, the thyroid and parathyroid, the stomach, and the small intestine showed no structural changes.

DISCUSSION AND SUMMARY OF RESULTS

The results of these experiments are interesting in that they show that very decided physiological reactions follow repeated intravenous injections of an active deposit of radium emanation. Clinical data have established the fact that external applications of radium or x-rays will produce decided changes in the composition and number of blood cells, and the results of this investigation show that the same changes may be obtained by injecting intravenously the active deposit of radium emanation.

In the case of external applications of filtered radium the physiological changes are produced mainly by gamma ray activity, while in the case of the radioactive solution used in the present experiments, the effect is largely due to alpha ray activity. The results show that following such intravenous injections there is a prompt reduction in the number of white blood-cells, which was especially well illustrated in the case of dog II, whose white cells were reduced from 14,400 before the treatments to 1400 at the end of the experiment. The terminal dose for the same animal also produced a remarkable reduction in the relative percentage of lymphocytes. As shown in table 2, after repeated doses of the radioactive solutions, the percentage of lymphocytes was reduced from 19 before treatment to 3 after the fourth injection. In this case, dog II, the four injections totaled 338.4 mc., and the last dose that preceded the sudden drop in lymphocytes, was a very large one, 146.4 mc. In both animals, however, after an initial moderate dose of the active deposit there was a gradual increase in the relative percentage of circulating lymphocytes in the blood. Dog I, that received 231.8 mc. in four injections (the total dose was 106.6 mc. less than the amount given to dog II), showed a terminal increase in the relative percentage of lymphocytes after moderate doses, which however were sufficient to reduce considerably the total white blood-cell count.

If it is true that following the instances where moderate doses of the active desposit of radium emanation were given to these animals there was an increase in, or a tendency to an increase in, the actual number of lymphocytes in the body, not merely those in the circulation, then these results may be considered similar to those of Murphy (3), of Murphy and Morton (4), and, more recently, of Murphy and Nakahara (5 and 6), in their experimental work concerning lymphoid destruction and the stimulation of the lymphoid elements in animals after exposure to x-rays. It is possible that such was the case, but the histological data at hand are not sufficient to settle the point.[1]

[1] In order to determine the presence of lymphoid stimulation it will be necessary to kill the treated animal after a single moderate dose, and not to continue with larger doses as was done in these experiments. Further experiments are planned to elucidate this subject.

The present experiments show that the apparent red blood-cell destruction was slight in comparison with the white blood-cell changes. In the case of dog I, see figure 1, an initial injection of 95.3 mc. of radioactive solution was promptly followed by a constant increase in the number of red blood-cells, which remained above normal until the second injection was given. This increase, however, may not have exceeded the limits of variability normal to the changes in the number of red blood-cells. After the second treatment, there was a sudden return to the normal number, followed by gradual increases in the red blood-cells, which continued to remain above normal from then on until the end of the experiment. Dog II lost about 25 per cent of its red blood-cells after an initial dose of 120 mc. This was no doubt a significant response. This loss was followed by a gradual increase after the injection of the two succeeding moderate doses of 17.3 mc. and 54.7 mc. respectively. The large terminal dose of 146.4 mc. again reduced the number of red cells to their previously low figure.

Comment on the subject of these red blood-cell changes brings up a point similar to one discussed in regard to the possibility of the stimulation of lymphoid production following intravenous injections of the active deposit solutions. Again the histological data are not sufficient to show the presence of increased red blood-cell production in the bone marrow, etc. How much of the apparent increase in the circulating red blood-cells was due to the attending diarrhea, or other undetermined conditions, is still unknown.

During the period in which the white blood-cells were being materially reduced there was a decidedly noticeable tendency for the blood to clot with considerable rapidity. This condition may have been brought about by the possible liberation of enzymes set free as a result of the destruction of large numbers of blood-cells.

In a previous work of the writer in which white rats were used as subjects, it was found that following lethal injections of the active deposit of radium emanation, the animals died after showing symptoms of marked enteritis. This subject was discussed,

and the phenomenon attributed to the fact that experimental data tend to show that a considerable amount of the radioactive substance was deposited in the intestinal tract. In the case of the dogs of the present investigation similar digestive disturbances were noted. After the first injection of 95.3 mc. dog I became inactive, diarrhea was present, and the dog refused to eat; but this condition lasted for only two days. The second injection was smaller, 30 mc., but following it the animal remained well and active, and showed no signs of digestive disturbances. The third injection consisted of 42 mc., and was followed by a slight amount of diarrhea, loss of appetite, and a reduction in weight. The fourth treatment, consisting of 64.5 mc., resulted in the animal refusing its food altogether, severe vomiting, and a further loss in weight.

The reaction of dog II to an initial dose of 120 mc. was interesting in that no digestive disturbances were noted until three days after the treatment, when vomiting occurred, and the animal became dull and inactive and would eat only a part of its food. The following injection consisted of 17.3 mc., and, as in the case of the small injection for dog I, it produced no digestive disturbances. Even with a dose of 54.7 mc., the third for dog II, no physical changes were noted, except that the animal was somewhat inactive for three days, and refused to eat on the fourth. The terminal large dose, however, 146.4 mc., produced a prompt reaction in the case of dog II. Vomiting was severe, a large amount of mucus covered feces was extruded, the animal refused to eat altogether, and lost two pounds in weight within five days.

Similar, but not as severe, intestinal disturbances were noted by Berg and Welker (7) in their work on the metabolism of dogs in nitrogenous equilibrium. They found, however, that no gross symptoms, except diarrhea, resulted from the administration by mouth of a preparation of 240 activity of radium bromid.

As shown in figure 2 and figure 4 the limits of the temperature changes indicate a considerable daily variation. It is thus difficult to make any definite statements concerning temperature reactions in response to this type of radium treatment. The sharp

rise in temperature in response to the terminal dose that was given to dog I is no doubt beyond the limits of the probable error of the variability for that case, and indicates a definite attempt on the part of the organism to adjust itself to the toxic disturbances brought about by the radium treatment. This toxemia probably resulted from the relatively sudden destruction of a large amount of cellular material which had to be eliminated by excretory organs, already damaged by previous radium treatments and passing through processes of degeneration. A similar temperature reaction probably holds good in the case of the second treatment for dog II, although the dose in this case was a small one, and again in the terminal reaction for the same animal, although why the treatment should be immediately preceded by a high temperature as well as followed by one, is still unanswerable.

The effect of repeated injections of an active deposit of radium emanation upon the weight of the animal is clearly shown in the case of dog II, see figure 3. There was a gradual almost constant decrease, amounting to about 25 per cent of the original weight.

The metabolic changes showed in the main that the total nitrogen content of the urine, the urea, the creatinine, the uric acid, and the total phosphates were markedly increased, probably as a result of active tissue destruction, and also to some extent due to the interference of the proper functioning of the excretory organs, as a direct result of the injections of the active deposit.

That active degenerative changes had occurred as a result of the radioactive injections is shown by an examination of the microscopical sections of the organs. These results resemble those obtained by the writer after similar injections in white rats. In both experiments there was considerable congestion in the principal organs, but the more pronounced degenerative changes in the white rats were far more severe than those that occurred in the dogs.

As previously stated in detail, a considerable amount of degeneration was found in the liver and kidneys of the animals. The spleen was also considerably altered, while it is to be noted that the bone marrow from the head of the femur in dog II was

devoid of lymphoid cells. The lymph-nodes as well as the lungs showed pathological changes in the case of dog II. The intestinal tract of dog II apparently showed no definite changes, while in the case of dog I, the only pathological changes that were noted were found in the colon, which was markedly congested, and showed signs of active mucus production. The stomach, thyroid, pancreas, and musculature presented no definite changes.

From a consideration of the results of the investigation as a whole, one may say that in the intravenous use of the solution form of the active deposit of radium emanation, as a therapeutic agent in cancer or other diseases, it is necessary to keep in mind the fact that after an initial dose of such radioactive substance the animal organism is irretrievably altered, and from then on will not give the same reaction to a repetition of the initial dose. The organism, as seen in the dogs of this experiment, is able to compensate for a severe initial dose of the radioactive substance, but after a certain point has been reached the natural protective adaptations on the part of the organs affected become inadequate to meet the demands of the organism as a whole, and the effects of the intoxication are greatly aggravated.

CONCLUSIONS

1. Large intravenous doses of an active deposit of radium emanation produce a considerable reduction in the number of white blood-cells, but while the white cells may be reduced by as much as 80 per cent of their total number, from the effects of an initial dose, the simultaneous reduction in the number of red blood-cells is less, and amounts to a reduction of about 25 per cent.

2. Repeated doses, amounting to a total of 338.4 mc. distributed in four intravenous injections, apparently produce a very marked decrease in the number of circulating lymphocytes of the blood.

3. Digestive disturbances, such as severe vomiting and diarrhea, followed large doses of the radioactive solutions, and were associated with a considerable reduction in the body weight.

4. In several cases, a rise in body temperature followed the treatments, suggesting an adaptive reaction on the part of the animal organism to meet the toxic condition produced by the relatively sudden destruction of a considerable amount of cellular material.

5. The metabolic changes, as determined by the daily urine analysis showed that the larger injections of the radioactive solutions were followed by very decided increases in the total nitrogen content of the urine, the urea, creatinine, uric acid, and the total phosphates.

6. Relatively small or moderate doses of the radioactive solution, if administered after the organism has already been injured by a previous injection, even when the metabolism is again back at normal, produce definite changes in the chemical content of the urine, indicative of destructive changes within the organism. These injections may or may not be followed by gross symptoms of digestive disturbance.

7. The histological study of the organs showed considerable congestion in the principal viscera. The liver showed a general fatty and granular degeneration, the kidneys showed a granular degeneration of the tubule cells, and the spleen presented considerable congestion, while the splenic pulp was largely drained of cells. The bone marrow was devoid of lymphoid tissue, which was largely replaced by fat. There was extreme congestion in the pulp of the lymph-nodes in one animal, associated with a reduction in size of the lymph-follicles. The lungs in one animal were normal, while in the other, which received the larger total of the radioactive solution, they were congested and showed slight signs of emphysema. The colon in one of the animals was markedly congested, and showed signs of active mucus production. The other organs showed no definite changes.

8. When the active deposit of radium emanation is used intravenously as a therapeutic agent, great care should be taken to grade the dose in accordance with the general physical condition of the patient, which should be determined by frequent urine and blood analyses; and if more than one dose is given during the treatment, the second dose should be made smaller in accordance with the strength of the original dose.

ACKNOWLEDGMENT

The writer wishes to express his indebtedness to Dr. James Ewing for his aid in the interpretation of the pathological results, and to Miss Ruth Theis and Dr. Stanley Benedict, who planned the chemical scope of the experiment.

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A STUDY OF THE OXIDASE REACTION WITH A-NAPHTHOL AND PARAPHENYLENDIAMINE IN TUMORS

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From time to time biological studies dealing with color reactions obtained in tissues and due to oxidation have been reported. These so-called oxidase reactions may be obtained by many different substances, belonging mostly to the phenols; among them are the two employed in this study. The development of color in the reagent depends primarily upon changes in its surface properties inducing oxidation, and these may be modified by contact with different tissues.

The various gradations of color indicate the amount of oxidation occurring, and since oxidation involves the acquisition of positive charge the tissue reaction is an index of the available positive charge which can be transferred by that tissue. In the following pages it is shown that the degree of color can be either increased or decreased within certain tissues. This we believe is closely related to potential of tissue. The less susceptible the tissue is to the reducing action of the reagent, the smaller the amount of color appearing and vice versa. If the conditions of the experiments are kept comparable variations of color in any one tissue, therefore, become a measure of the alterations in the potential energy of the tissue in question.

The early literature of the occurrence of oxidases in animal tissue deals almost entirely with the polymorphonuclear leucocytes and their precursors, the myelocytic cells and it is only in recent years that the study of the oxidase reaction has been extended to tissue other than white blood cells. It is not surprising,

therefore, that the work reported on the oxidase reaction in tumors is not extensive. We have been able to find in the literature only three references to the occurrence of oxidase in tumor cells, and in two of these the tumors were derived from blood elements. Thus in 1917, Forman and Warren (1), accepting the synthesis of indophenol blue in tissue as diagnostic of myelocytic cells, studied the distribution of the oxidase reaction in a myeloma by means of a reagent consisting of 1 per cent α-naphthol in a 1 per cent solution of KOH with 1 per cent dimethylparaphenylendiamine. They observed color formation in the greater number of the cells of which the tumor was composed. Boots (2), working in this laboratory, obtained a positive reaction in a case of chloroma with the Graham modification of the oxidase reagent but a negative finding when he employed the Schultze technique.

Graeff (3) in 1912, however, had mentioned the occurrence of oxidase granules in adenoma and carcinoma (page 380) though he failed to find any trace of the reaction in a spindle-cell sarcoma of the kidney. In the same article, he adds that von Gierke had personally communicated to him the finding of a distinct oxidase reaction in the cells of mouse tumors.

Recent work on normal histological material by several investigators has demonstrated the wide-spread distribution of the oxidase reaction. The occurrence of the reaction in varying degrees in practically all normal tissues of the body suggests its application to pathological material. Further, since the reaction has been shown to be an adsorption phenomenon, which is dependent on such factors as the composition and potential of the various reacting components, it was thought possible that a study of tissue from neoplastic growths might yield some data concerning variations of the surface properties of the cells or their constituent elements.

TECHNIQUE

The material used in this study was derived from surgical specimens removed at operation. These had been preserved in 5 per cent formalin and form part of the pathological stock ma-

terial. In a few instances results were checked by sections made from recently obtained material. By repeated washing in distilled water the excess of formalin was removed from the blocks of tissue, which were cut by the freezing microtome in sections varying in thickness from 5 to 10μ. These sections were then immersed for varying periods of time in a reagent composed of an aqueous solution of M/2000 α-naphthol and M/2000 paraphenylendiamine. The details of the staining reaction and technique have already been discussed in a previous article (4). It may not be amiss, however, to emphasize again the precaution of keeping the amount of tissue small in comparison to the amount of reagent in which the sections are immersed. As already noted, formalin fixation has no appreciable deleterious effect on the development of the oxidase reaction in the tissue. However, Zenker's fluid alters the tissue so greatly that after fixation in this medium the reaction fails entirely or obtains only in a very small degree. Not infrequently as a result of formalin fixation, deposits of unknown composition are encountered in the sections, and as these may sometimes interfere with the recognition and interpretation of a positive oxidase reaction, unstained sections were always mounted in the later part of the work for purposes of comparison with the stained. Sections were mounted in a 50 per cent aqueous solution of glycerin. The tissue thus treated can be preserved fairly well for several months. The time of immersion required to produce color reactions varies usually from three to twenty hours, though changes in the functional activity of the cell, as will be pointed out subsequently, exert a marked influence on the rate of reaction, so that frequently one to two hours suffices to produce a maximum color. We have restricted our study to well-defined types of neoplasms in which there was no question concerning the diagnosis.

TUMORS ARISING FROM CONNECTIVE TISSUES

Corresponding to the numerous modifications occurring in the morphology of normal connective tissues is an almost equal number of types of tumors arising from connective tissue. We have studied the oxidase reaction in sarcoma, fibroma, lipoma,

osteo-chondroma, and myxoma. That normal connective tissue does not readily react with the oxidase reagent is indicated by the negative findings of Wolff (5) on connective tissue of placenta when sections of this organ were stained with a 1 per cent aqueous solution of α-naphthol and paraphenylendiamine and by the somewhat contradictory statements of von Gierke (6) and his pupil Graeff (3) regarding the ability of connective tissue to stain with the above reagents when dissolved in normal salt solution. von Gierke, using a combination of these two substances in 0.75 per cent concentrations, reports a positive finding, while the latter, with a more dilute concentration of the above reagent, failed to obtain any reaction except an occasional granule at the edge of the nucleus. Graeff believed the presence of a positive reaction to be dependent on the age of the cell. As has been previously shown by us the oxidase reaction does not readily develop in connective tissue in a watery solution of these substances. After an immersion varying from ten to twenty hours in an aqueous mixture of M/2000 α-naphthol and M/2000 paraphenylendiamine (and it should be emphasized again that α-naphthol is not soluble in cold aqueous solution above M/2000 concentration and even at this concentration occurs in the colloidal state) a blue color obtains in the protoplasm lying immediately about the nucleus, and especially at either pole. This blue color is due to the presence of fine granules. Coincident with the staining of these perinuclear granules there was found a blue staining of the nucleolus and frequently, though not always, the chromatin knots showed a faintly positive reaction. The color produced in these perinuclear granules may be considerably varied by modifications in the reagent, and can be enhanced by the addition of certain salts in definite proportions. Thus using the Sörensen phosphate series as a solvent for the α-naphthol and paraphenylendiamine, it was previously shown that these perinuclear granules reached a maximum size and number and developed the most intense color at a concentration corresponding to about P_H 7.46. Undoubtedly, not only the composition of the media bathing these cells but also their functional activity exerts a considerable influence on the degree of the re-

action. As color development depends upon factors governing adsorption, fixation of tissue as well as concentration of reagents and composition of its solvent will modify its rate. In the normal connective tissue cells these perinuclear granules possess some degree of lability. This property is more evident in certain tumors of connective tissue derivation.

Sarcomata

The reaction as a whole obtaining in sarcomata is characterized by the negative or relatively slight reaction in the nucleus and the contrasting degree of the stain occurring in the granular cytoplasm immediately surrounding the nucleus. In actively growing spindle shaped cells in fibrosarcoma there occurs a considerable deposition of the indophenol blue upon the perinuclear granules and especially in the region of the poles of the nucleus. This granular material appears to be somewhat greater in amount than in the normal cell and in many of the tumor cells is found extending in cone like formation almost to the spindle ends of the cells. Between the nuclear wall and these stained granules, which are relatively fine in size, no clear zone can be discerned. Indeed, the reaction is most intense in the cytoplasm in the region immediately contiguous to the nucleus. The nucleus in these actively growing fusiform cells usually gives no reaction whatever though occasionally a very faint violet tinge may be observed in the nucleolus. The nuclear wall appears as a mauve outline, but definite granules cannot be distinguished. I have never been able to recognize chromatin threads or knots in the nuclei of these cells. The contrast between the practically colorless nuclei and the mauve granular cytoplasm is striking. In the cells with the nuclei of a more spherical form the granular reaction is fairly uniformly distributed in a narrow zone about the periphery of the nucleus. When giant cells occur in the sarcoma the reaction is deeper in color than in the smaller cells and it is almost impossible to distinguish individual granules because of their close aggregation. Frequently this colored granular material is so densely packed as to obscure the nuclei, which are color-

less. It is not clear whether the greater intensity of color results from an increased number of granules within the cell, or from a deeper color of individual granules.

The most remarkable reactions which occur in sarcomata, however, are to be observed in those cells which through lack of adequate blood supply show degenerative changes. Under these altered conditions of environment and nourishment the perinuclear granules have become so altered in composition and surface properties that with a short immersion in the oxidase reagent one obtains colored granules which rival and even surpass in intensity those found in the myelocyte. These degenerative changes can be followed most readily in the spindle shaped cells. The alteration in the staining qualities of the cell appear first in the granular protoplasm about the poles of the nucleus, where the degeneration manifests itself by an increase in the size and number of the granules, which also assume a more intense violet color. As the degeneration process progresses, the color gradually increases in intensity and finally becomes blue-black. Through a coalescence of the smaller particles there result many large granules, which spread not only out toward the pointed ends of the cells but may also extend over the region of the nucleus. Coincident with these cytoplasmic changes the nucleolus takes on a bluish tinge, and finally stands out as a dark blue mass. Fine chromatin lines with irregularly distributed deeper masses representing chromatin knots can also be distinguished. The nuclear wall has become much deeper in color and definite granules appear on its surface. It is difficult to determine whether or not the nucleus participates in the formation of these extra-nuclear granules. Finally the whole cell may be filled with these deeply blue stained masses of varying sizes, many of which have become incorporated into large globules which appear to possess a semi-fluid consistency. The Brownian movement which these particles possess is very apparent in these large globules. The oxidase deposit occurs only on the periphery of the large globules. In the cells of oval and circular contour the degeneration may begin at any point, and progress to the final stage in which the cell protoplasm

has been largely converted into a mass of colored globules. These globular masses can be recognized in the formalin fixed unstained frozen sections, and can also be stained with neutral red. They appear to be identical with the granules in fibroblasts grown in vitro, which have an analogous distribution and which have been recently described by Lewis (7). The intimate association of these granules with the centrosomes and mitochondria has already been noted by that author. In melanosarcoma the granules giving the oxidase reaction are quite distinct from the melanin pigment of the cell. The degenerative changes just outlined, which are accompanied by the increased affinity for indophenol, may develop in these pigmented cells and apparently have no relation whatever to the melanin granules. In lymphosarcoma the nucleus is relatively free from reaction and fine deep blue granules appear irregularly scattered throughout the cytoplasm. The appearance is similar to that previously described by us for the normal lymphocyte. When the sarcoma cells in the course of their development give rise to intercellular collagen, this substance stains a diffuse pink with the occasional formation of more deeply reacting longitudinally arranged fibrils. Besides the changes in the sarcoma cells, others may occur in the same sections throughout the normal tissue; these consist of large deposits of deeply stained material resembling fat and occupying the connective tissue framework. Occasionally these may be displaced leaving cavities. These globular masses stain on the periphery only and not infrequently the center consists of numerous unstained white acicular crystals.

Fibromata

Oxidase reaction was studied in six fibromata, all of which had arisen in the skin. Like sarcomata, these are characterized by the very faint reaction occurring in the nucleus, and the more intensely staining zone of perinuclear granular material. Two of the tumors were of the *fibroma durum* type. In these the nuclei were small and the perinuclear granular protoplasm much reduced in amount. The nuclei showed no reaction except for

a very faint mauve coloration of the nucleolus. Lying about the nuclei and extending longitudinally outward from either pole as a thin line there is a very intensely stained, blue-violet, granular protoplasm. This narrow strip lies between thick parallel bands of collagen which for the most part is homogeneous and stains a pale pink, although in some areas it may show the presence of faintly mauve colored granules, which occasionally are arranged in fibrils lying parallel to the long axis of the nucleus. Other histological structures occurring in the skin, e.g., sweat and sebaceous glands, hair follicles, and malpighian layer give a very intense reaction.

In the other four tumors, which represent various degrees of the soft type, the cells are larger and the matrix less in amount and in these the reaction approaches more closely that of the fibrosarcoma. The nucleolus can be distinguished as a mauve stained body, and occasionally chromatin knots may be faintly recognized. The karyoplasm stains a pale pink and under high magnification faint granules may be made out.

In two of the sections there occurs a considerable infiltration of lymphocytes and plasma cells, the cytoplasm of both of which shows numerous deeply stained moderate sized granules. Areas of these cells alternating with the more lightly reacting connective tissue elements afford a marked contrast. Degenerative changes comparable to those described in sarcomata are apparently not of frequent occurrence in fibromata, as in only one of the tumors studied was there any evidence of a degenerative process giving rise to those intensely reacting granules which are of such constant occurrence in sarcoma. The degenerative processes in the two types of tumors resemble one another closely. In fibroma, however, the nucleus appears irregular in outline and the nucleolus and chromatin knots assume a deeper stain at an earlier stage of the degeneration. The more deeply reactive globules appear first in the region bordering the poles of the nucleus. They may vary in size and may attain very large dimensions, and in the late stages appear irregularly distributed throughout the cell contents. Occasionally these deeply stained spherical globules may be extracellular.

Myxomata

Only two cases of myxoma were studied. The sections consisted of a pink stained homogeneous matrix, in which were irregularly scattered many cell bodies with fine thread-like prolongations ramifying in all directions.

The cell body consisted of numerous deeply stained granules embedded in a homogeneous mauve protoplasm. The granule formation extended outward into the prolongations, which appeared of a deep blue color owing to the fact that the granules lay closely packed together. The nucleus showed no reaction except for a faint diffuse mauve color in the nucleolus which was often obscured by the wealth of overlying deeply stained granules.

Osteo-chondroma

Only two sections were studied. At the periphery of the tumor, where proliferation was active and where the cells were fusiform in outline, the reaction within the cell was most intense at either pole of the nucleus, over which it extended like a cone. The cytoplasm consisted of a pale mauve matrix in which were embedded many deep blue granules. At the periphery of the cell in the region of the cell membrane these became denser and more closely packed together. The nucleus showed a fairly well stained nucleolus, but it was not possible to distinguish chromatin.

In the zone lying next the periphery of the tumor, where the cells were still rapidly dividing, many cells were arranged in pairs with two sides in apposition as in normal cartilage. In these the reaction was similar to that occurring in the cells already described. The matrix in which these cells lie was of a faint homogeneous pink color. In the more mature parts of the tumor the cells were larger in size and the granules lying in the cell body became coarser, more deeply stained, and frequently assumed a concentric arrangement about the nucleus, where they sometimes occurred in such close apposition that it was difficult to recognize individual granules. In the same fields many cells were also found in which the ground substance of the cell had become almost colorless, but throughout this colorless protoplasm were

scattered many coarse deep blue granules. The periphery of both types of cell was formed by a narrow zone of coarse intensely stained granules. The matrix in which these cells lie was for the most part homogeneous and more deeply stained than near the periphery of the tumor growth. In certain areas it was converted into reddish stained fine fibrils, irregularly scattered through which one could find larger masses of deeply stained material.

Lipomata

The four lipomata studied belong to the subcutaneous type. In two of them the supporting stroma had been much reduced and the cells were so distended with fat that the tumors were difficult to section. In staining, the contents of the cells of the lipoma gave a reaction which could not be distinguished from that observed in cells of normal adipose tissue. In those cells where the conversion into fatty material had not been complete, the small globules of fat stained readily and appeared almost black in color. In cells which were so loaded with fat that the cytoplasm and nucleus could no longer be recognized, the lipoid contents of the cell stained on the periphery only and were of a deep blue-black color. In many of these large cells the central part was composed of long acicular crystals, which did not react with the oxidase reagent. In one of the sections orange yellow fatty deposits occurred, which stained much more slowly than the cells containing colorless lipoid material. The normal structures of the skin associated with the lipoma gave reactions similar to those found elsewhere in the skin.

Myomata

The myomata studied belong to the leiomyomata of the uterus. The oxidase reaction found in the muscle cells of the uterus occurs with greatest intensity in the undifferentiated protoplasm lying about the nucleus. This small area of granular protoplasm can be stained by other reagents and the granules have been demonstrated by Lo Cascio (8) with iron hae-

matoxylin. Their staining properties apparently are closely related to their functional activity, for both Graeff (3) and Ikeda (9) found that in the muscle cells of the pregnant uterus the oxidase reaction developed more rapidly and with greater intensity than in the muscle cells from the normal organ. These observations on the variability of the reaction with the oxidase reagent we have been able to confirm and have noted marked differences between the staining of the myometrium from different preparations. It may vary from a mauve reaction in which the granules can not readily be recognized, to distinct granules with a maximum blue-black color. In one case, in which an adenocarcinoma had invaded the myometrium, a few minutes following immersion of sections in the oxidase reagent the appearance of blue color was observed in the perinuclear granules of the muscle cells in areas irregularly distributed, and at the end of from two to three hours a reaction equal in intensity to that of the myelocytes had developed. It has not been possible thus far to determine definitely the factors underlying these variations. The fact that they do not occur uniformly distributed, and that the areas in which the reaction is most intense are bands of myometrium which have become segregated by the actively growing cancer cells, suggests decreased metabolism through pressure to be a predominating factor.

We have found that in myomata the perinuclear granules always show a pale mauve color at the end of five or six hours, and at the end of twenty hours stand out fairly distinctly. Simultaneously with staining of the cytoplasmic granules, color appears in the nucleolus and chromatin knots. The distribution of the extra-nuclear oxidase reaction seems to depend largely upon the shape of the cell. In the spindle shaped cells these granules lie as in the similarly shaped connective tissue cells, aggregated about either pole of the nucleus. They may lie in close apposition to the nucleus or may be separated from this structure by a narrow clear zone. In these cells the granules are usually of fairly large size and are intensely stained. While the distribution is similar to that of the fusiform connective tissue cells, the two can be differentiated by the difference of the

intensity of the reaction of the perinuclear granules and of the nucleus. In the connective tissue the reaction in the nucleus is almost negative in comparison to the decided mauve color of the nucleolus and chromatin material of the muscle cell, and in normal tissue the perinuclear granules of the muscle cell always stain more deeply when immersed in the same reagent for like periods of time than the corresponding granules of the connective tissue cell. When the cells and nucleus approach the more circular form or are oval, the extra nuclear material appears uniformly surrounding the nucleus. This granular material may lie contiguous to the nuclear membrane or may be separated from it by a clear zone. The amount of this material is never very large and may be present in amounts varying from a few granules to 20 or 30 or to a cloud of intensely stained fine granules. Graeff (3) has also noted considerable variations in the number of the perinuclear granules of the normal muscle cell which give the oxidase reaction.

The granules vary in size and in some of the sections examined granular formation could only be distinguished under high magnification. When fibromatous elements are present in the myoma, the longitudinally arranged collagen bands reveal a pale pinkish coloration, in which occasionally fine fibrillation can be seen. The reaction in the nucleus consists of a number of irregularly scattered, large sized, well stained granules. The nucleolus also stains deeply. In some of the preparations the granules are imbedded in the nuclear wall, and they vary somewhat in size. The reaction appears first in the nucleolus but also develops early in the chromatin knots.

Myeloma

Only one myeloma was available and this was of the myeloid type. The gross specimen, which consisted of various sized masses of soft yellowish brown material was curetted from the upper part of the tibia. On microscopic examination the section was found to be fairly vascular, and composed of closely packed medium sized cells with a large clear nucleus and a com-

paratively small amount of cytoplasm. Irregularly scattered throughout the section also were giant cells, containing from three to ten nuclei. These cells gave the sections a very characteristic appearance. They exhibited a marked affinity for the indophenol, showing the presence of a definite bluish color following an immersion in the reagent of from one to two hours' duration.

At the end of seven or eight hours they stood out as intensely blue stained masses. The cytoplasm was homogeneous and mauve in color and in it were embedded deep blue granules of varying size, which were closely aggregated. The nuclei were colorless and morphological detail was obscured by the overlying granular material.

The smaller cells had colorless nuclei. The cytoplasm also was colorless, and contained coarse deep blue granules, in the larger of which Brownian movement could be detected. In addition to the oxidase granules these cells occasionally showed the presence of globular masses of yellowish material, which did not react with the reagent.

Endotheliomata

In a previous paper we (4) reported a very slight pink reaction obtained in the endothelium of blood vessels, a finding which is in accord with the observations of von Gierke (6) and Graeff (3). Though more than twenty tumors of blood-vessels have been examined, among which were endotheliomata and lymph- and haemangiomata, we have invariably found that the endothelial cells of these growths also exhibit a negative or faintly positive reaction.

TUMORS DERIVED FROM EPITHELIAL TISSUES

Normal epithelium everywhere throughout the body reacts to a greater or less degree with the oxidase reagent. The color formation which is obtained varies with the organ and the part of the organ in which the epithelium occurs, as well as with the physiological activity. Thus Klopfer (10) reports various gradations of the indophenol synthesis in different parts of the kidney

tubule, and Lillie (11) finds that while the epithelium throughout the intestinal tract gives a positive reaction this is most intense in the stomach. Similarly we (4) have observed the most intense reaction of squamous stratified epithelium in the skin. The influence of functional activity upon the oxidase reaction is well illustrated by the varying degrees of intensity obtaining in the secretion granules during the different stages of the secretory process in the intestinal tract. The same wide variation found in normal epithelium is also found in tumors of epithelial derivation. It is noteworthy that it is those tissues showing wide variations in the oxidase staining capacities in which the cancer incidence is high. That is, tissues in which the lability of the granules (as measured by differences in the oxidase reaction) is marked, are those in which tumors are prone to occur. Among these one may especially mention stratified squamous epithelium and epithelium of the intestinal tract and the uterus.

The oxidase reaction has to do primarily with the surface properties of protoplasmic granules and one may obtain the same picture in cells from tumors which vary widely in structural characteristics. Cells in various areas of the same tumor may show the widest divergence; cells from different types of tumors in the same or in different organs may appear identical in staining qualities. Thus while considerable difference occurs in the morphology of papillary and adenocarcinoma the cytology of the two may be indistinguishable.

As has been already indicated, the range of the oxidase reaction within different organs or different parts of the same organ is not the same. In normal structures this range of intensity is largely dependent upon the intrinsic constitution of the part and its physiological activity. In the tumor one recognizes that in addition to these two factors the age of the cell is also a potent influence in modifying the development of color. That is, in cancer, the intensity of the oxidase reaction closely corresponds to the stage of cell development, being minimal in the newly formed cell and gradually increasing until a maximum is reached in the old degenerating cell. Thus, in the peripheral parts of the cancerous growth, regardless of its structural peculiarities, the

outstanding characteristic is the ability of the rapidly dividing cells to inhibit the indophenol synthesis compared with that displayed by the cells of the maturer, better developed tissue. It is evident from the above that any very detailed account of the oxidase reaction in carcinoma would consist largely of a description of color reaction of varying intensities. As this study deals with reactions in individual cells rather than structural differences, to avoid repetition and make descriptions as concise as possible we have arbitrarily divided cancer into two groups according to whether they are derived from stratified squamous epithelium or glandular epithelium.

Carcinoma Derived from Glandular Epithelium

Under this heading are included growths derived from the epithelium of the intestinal tract below the oesophagus (consisting mostly of those of stomach and rectum) and from the uterus and breast. They vary in architectural structure and may be of adenomatous, papillary, or diffuse types.

Where the tumor is actively proliferating the cells appear of a very pale mauve color. This is partly due to the fact that the nucleus, which is large in proportion to the cytoplasm, is very faintly stained. The nucleolus is barely discernible and the remainder of the nucleus is made up of a network of very faint mauve lines in a colorless karyoplasm. The nuclear membrane is seen as a thin, deep blue line. The cytoplasm is scant and is filled with granules of a faint reddish mauve color. As the cells become more mature the granules increase in color and the nucleolus and chromatin threads become more prominent, taking on a bluer tinge. As the cells tend towards degeneration the nucleolus assumes a swollen appearance and becomes brownish in color, and the chromatin threads remain fairly well stained.

The gradual increase in size and color of the protoplasmic granules is well illustrated in the goblet cell where, as the mucous secretion accumulates, the granules gradually become larger and acquire a greater affinity for the stain. In the rectum one may occasionally find accumulations of these deep blue granules lying

immediately over the mouths of the goblet cells. The nucleus shows a faintly stained chromatin network with a fairly deep mauve nucleolus. When the growth, becoming highly invasive and, losing its adenomatous character, grows diffusely, as in the uterus for example, the granules in the cytoplasm may stain a deep blue and be so numerous as to obscure the histological details of the nucleus. In the breast, when the adenomatous character of the growth persists, the gland epithelium contains a few coarse granules near the base of the cell, where the nucleus occurs, but a wealth of fairly coarse granules occur within the free border. The connective tissue, when it has not been encroached upon to any extent by the surrounding neoplasm, shows a fairly normal reaction. When the tumors, however, proliferate so rapidly as to give rise to pressure upon the interstitial tissue, the perinuclear granules of the connective tissue cells may show a very distinct deep blue reaction, due to the formation of degeneration granules. This phenomenon is common in tumors of the breast, but is exhibited in its greatest extent in the colloid cancers of the stomach. In rapidly proliferating tissue in which the nuclei became much enlarged and contained chromatin in clumps, we have noted that these latter exhibited a marked affinity for the indophenol and appeared as blue-black masses.

In those adeno-carcinomas of the stomach and rectum which are designated "colloid cancers" because of the hypersecretion and subsequent accumulation of mucin in the alveoli, the substance secreted undergoes a change in its staining qualities subsequent to its expulsion from the cell. As it lies just outside the lumen of the gland cells it consists of many very deep blue granules embedded in a pink homogeneous material. When the alveoli have become distended with this mucous secretion, however, the granules tend to disappear and the matrical substance to become colorless. This decoloration is most apparent in the central part of the alveoli.

Squamous Cell Cancers

In general the oxidase reactions obtained in the stratified squamous epithelium which has acquired carcinomatous properties closely resemble those found in the normal epithelial tissue of a similar type. This layer, not only where it is limited to a normal distribution, but also where its outgrowths have invaded the underlying tissue, stands out in sharp relief against the more faintly reacting contiguous connective tissues as a deep blue mass. In areas where keratinization has taken place, both in stratum corneum and in the epithelial pearls, the oxidase reaction reaches its maximum intensity and appears as a dense blue-black mass. It may be so intense as to obscure all morphological detail.

In contrast to this the stratum lucidum when present shows a very pale reaction. Within the epithelial growth the constituent cells in the various strata exhibit considerable variation in staining qualities. At the growing tip of the cancer mass which is extending into the surrounding tissue, the reaction assumes a fairly intense diffuse color and especially in those cells which are rapidly dividing. This intensity of color is due to closely aggregated bluish-red granules in the homogeneous mauve cytoplasm, which is small in amount. The nucleus is comparatively large, and is clearly outlined by a fine deep blue nuclear membrane. In the colorless or faintly pink staining nuclear contents can be recognized the nucleolus, which is fairly large and is colored a diffuse mauve. Occasionally the chromatin knots appear faintly stained.

In the zone lying beneath that actively proliferating, the cells are larger; and while the individual granules are deeper in color because they are here not so closely aggregated, the whole cell may not show any increase in intensity of stain. In the layers more remote from the vascular supply the granules gradually become much coarser, of a deep blue color, and stand out very distinctly. The volume of the cells may be much increased, the nucleus is larger, and the nucleolus appears swollen and frequently may reach three or four times its original volume. It is much paler in color and usually appears to have a yellow-brown tinge. A chromatin network can be faintly distinguished.

As the cells become still more flattened and compressed the granules become progressively larger in size until at the centres of the epithelial pearls the entire cell contents are converted into large clumps of deep blue-black material. Occasionally what corresponds in staining qualities to the stratum lucidum may intervene beween the flattened keratinized cells in the centre of the epithelial pearls and the zone lying peripherally. These cells contain an almost colorless ground substance in which occur rather sparsely distributed granules, which stain a pale mauve. The nucleus is irregular in outline and almost colorless except for the presence of a large diffusely pale stained nucleolus.

It is of interest to note the reaction occurring in the fibroblasts, which have been stimulated to proliferation by the extension of the neoplastic cells into the connective tissue. Unlike the reaction usually found in the adult connective tissue or in fibrosarcomata, not only are the perinuclear granules deeply stained, but the chromatin as well as the nucleolus is stained. This increased color reaction is not infrequently observed in epitheliomata of skin and it is possible that pressure is a factor in its production. One also finds in these neoplasms structures which may occur in normal squamous stratified epithelium. Intercellular bridges when they occur stain a very deep blue in contrast to the pink homogeneous cement substance between the cells. These cellular connections are made up of very fine granules lying in close apposition. Further the fibrillation which is sometimes seen in the normal cell may be observed passing through the cytoplasm as parallel bands of fine blue lines.

Karyokinetic figures are never to be recognized as distinctly stained structures, but occasionally appear as faint shadows. In places collagen formation may occur, showing the usual pale pink stain with compressed deeply reacting perinuclear substance in the connective tissue cells lying between the longitudinal collagen bands. In the skin it may also be noted that where splitting of the elastic tissue occurs these fibres take on a diffuse deep bluish purple stain. An observation of interest was the deep reaction occurring in the medullary sheaths of the cutaneous nerves.

DISCUSSION

As has been frequently stated in this and in a previous paper on oxidases, if one employs a suitable combination of α-naphthol and paraphenylendiamine the oxidase reaction can be demonstrated in every tissue in the body, though not with an equal degree of intensity in all. As color development in the indophenol synthesis is due to oxidation it will be necessary at the outset to consider briefly the formation and composition of indophenol and part at least of the kinetics concerned in the production of its color, in order to obtain a better understanding of the staining process occurring in the tissues. As was indicated previously both α-naphthol and paraphenylendiamine form colloidal solutions in water. The colloidal particles of the former acquire in this medium a positive electric charge while the paraphenylendiamine becomes negatively charged. The sign of the charge on the colloids was determined by observing migration of these substances in a Michaelis (12) kataphoresis tube when an electric current was passed through the solution. Since the color of either substance alone in an aqueous solution is so slight, even when the electric current has been passing through a solution of one of them for a fairly long period of time and has effected the transport of a considerable amount of the colloid towards one pole, its presence in the region of this pole cannot be recognized by its color alone. If, however, the lower stopcocks of the apparatus are closed and the electrode tubes are carefully removed, addition of the same amount of paraphenylendiamine to the solutions in both arms of the U-tube, when α-naphthol is being studied, will indicate by the speedy development of color into which arm of the U-tube the colloid has migrated. Similarly if the migration of paraphenylendiamine is being investigated its charge can be determined by reinforcing the color by the addition of α-naphthol in equal amounts to both limbs of the U-tube subsequent to the passage of the electric current.

In aqueous solution, then, α-naphthol is an electro-positive colloid and paraphenylendiamine is electro-negative. Now if these two solutions are mixed, through the agency of surface

condensation there will result a new colloidal complex whose composition will depend in large degree upon the amount of neutralization occurring between the two oppositely charged colloids, which is determined by their concentration and degree of dissociation.

When these two colloids balance each other electrically a neutral compound will be formed and slowly precipitate out of solution. With this neutralization is associated development of color, as can be well demonstrated by employing the Sörensen phosphate solutions as solvents. When equal parts of the two substances composing the reagent are added in equimolecular (M/2000) proportions to the various solutions of the phosphate series, this electrically neutral compound, indophenol, is formed in greatest amount in the solution having a value of P_H 7.17, the so-called iso-electric point, since in the kataphoresis tube the colloid in a solution of this reaction moves to neither pole. At this point the maximum color develops. In the more acid or more alkaline solutions there is a definite migration to the positive and negative poles respectively and a corresponding decrease in color. In the solutions which are more alkaline in reaction than P_H 7.17 the diminution in color is less rapid than in the more acid solutions and little decrease in intensity is noted up to P_H 8.04. In aqueous solutions indophenol has a negative electric charge which is capable of being neutralized by surface condensation when contact with oppositely charged tissue constituents occurs. This is accompanied by increased development in color.

Now when sections are placed in an aqueous solution containing M/2000 α-naphthol and paraphenylendiamine the various tissue components acquire a negative or a positive charge or, if at their iso-electric point, no charge whatever. If sufficient ions are discharged into the water to alter its reaction to such an extent that the indophenol no longer reacts as a negatively charged, but has been converted into a positively charged colloid, oxidation does not occur and practically no color develops. This happens when the amount of tissue is large compared with that of water. If however a comparatively small amount of tissue be placed in the reagent the formation of the indophenol blue in the tissue

may be interfered with by a separate combination of either one of the reagents with the tissue components, or if the indophenol is formed, subsequent to its adsorption by the substances in the tissue, oxidation or reduction may take place. If the former process (i.e., separate reaction of the α-naphthol or paraphenylene with the tissue) occurs it is apparently not associated with the development of color in the tissue, for if one transfers the sections every hour to a freshly prepared reagent the development of the color is delayed indefinitely.

If, as in the second case, the indophenol is formed, whether or not color develops will depend upon the charge of the tissue component adsorbing this dye. If this is negative, reduction of the reagent or transfer of positive valence from it to the tissue occurs and no color is formed. If on the contrary the tissue is positively charged, there results through oxidation (or increase in positive valence of the reagent) indophenol blue, the amount of which depends upon the degree of dissociation of the adsorbing substance in the tissue.

Thus far we have discussed surface charge as if it were the only factor concerned in adsorption of indophenol. By means of a Traube stalagmometer it can be shown that the surface tension of a solution of α-naphthol is lowered on the addition of the paraphenylendiamine upon the formation of indophenol and its oxidation. While surface charge and surface tension are intimately related there is reason to believe that surface tension, per se, is an important factor in the formation of indophenol blue in the tissues. However, the relation of oxidation, which in its broad concept is analogous to an increase in the positive electrical charge, to alterations in color makes the connection between oxidation and surface charge easier of demonstration, than a definite relationship between oxidation and surface tension. The production of a marked color reaction upon immersion of normal tissue in the oxidase reagent in stratum corneum, fat globules, eosinophiles, neutrophiles, and decidual cells marks these as tissues possessing a common property which is related to electrical dissociation. The electro-positive character of fat globules and the granules of the stratum corneum is not so prominent as

their low surface tension. An evidence of the close relation which this latter property bears to the formation of a positive oxidase reaction is seen in the markedly enhanced reaction occurring in the lipoid substances of the medullary sheaths following the exposure of peripheral nerves to ether, chloroform, bile, and various other fat solvents which lower surface tension.

In contrast to the above enumerated tissue constituents showing a marked positive reaction are those in which a minimal reaction obtains. Among these one notes the nucleus preeminently but likewise the matrix of cells, collagen, and endothelium. Between these two extremes lie tissues in which all gradations of color appear.

The intensity of the color reaction in a tissue is thus a direct index of the amount of dissociation giving rise to a positive surface charge which the indophenol is capable of abstracting from the tissue. That is, the color reaction is a measure of the degree of reduction of tissue effected by the indophenol (or, what amounts to the same thing, is an inverse measure of the oxidative capacity).

Under certain conditions the reaction may also yield further information. It may constitute a gauge of the inherent potentiality of the tissues as well as the conditions and approximate rate by which this potential may be transformed into kinetic energy. This is illustrated in the variations of the reactions described in sarcoma. In the connective tissue cell under changes of environment, among which increase in the hydrogen ion concentration of the surrounding media is prominent, the finely granular positively charged perinuclear material is converted into colloidal complexes with new physico-chemical properties. The original granules, probably through the adsorption of other colloids, have been finally reduced to large globular masses with a low surface tension and considerable electrical charge. At this stage the oxidation power of the indophenol is high compared with that of the tissue granules and color formation in the former reaches a maximum.

By means of this reaction it can thus be shown that these perinuclear connective tissue granules not only possess a high

potential energy, but that this energy is capable of being readily transformed under pathological conditions into the kinetic form of energy, which readily reacts with the indophenol. Similar physiological transformations are also encountered in the conversion of the granular contents of the basal cells of the malpighian layer into the intensely staining eleidin and keratohyalin. It has even been postulated by Tettenhamer (13) though without very convincing proof, that in this instance the alteration actually includes the chromatin of the nucleus. It is an interesting coincidence that the tissues in which the lability of the granular contents, as demonstrated by the oxidase reaction, is most marked, are those in which the regenerative capacities are highest. While this lability of granule can be most readily demonstrated when it results in an increased oxidation of the oxidase reagent (or conversely a reduction of the tissue component) there is unmistakable evidence of its existence in the opposite direction. That is, an accumulation of potential energy is manifested in the nucleus and perinuclear material of actively proliferating tissue by the diminution in the intensity of their oxidase stain. Instances of nuclear change represented by increased development of color, and interpreted as a decrease in potential energy have also been described throughout the paper.

In conclusion it is to be emphasized that all the color reactions discussed in this paper are always in relation to the iso-electric point of the indophenol, which is fundamental, and hence the surface charges are not absolute but relative. Therefore whether oxidation or reduction predominate in tissue or reagent, depends upon the physico-chemical properties of each, which is determined in no small degree by the character of the media in which they occur. A slight alteration in the environment of amphoteric substances, especially in the vicinity of their iso-electric point, will induce far reaching changes in their reactions, as is attested by the conversion of positive into negative oxidase reactions, or vice versa, by the addition of traces of an alkali to the reagent.

SUMMARY

1. Development of color in the reagent is due to oxidation. Therefore, when the adsorption of the reagent by a tissue is followed by an increase in the intensity of the color, increased oxidative capacity has been developed in the reagent. This involves a corresponding tissue change in the nature of a reduction.

2. The more marked the oxidase reaction the greater the reduction occurring in the reacting tissue, and vice versa.

3. Cell constituents of certain tissues, in which a slight oxidase reaction normally develops, may through degenerative processes be converted into new colloidal complexes in which a maximum reaction obtains. Examples of such alterations in intensity of reaction are found in connective tissue (spindle-cell sarcoma) and in stratified squamous epithelium (epithelial pearls of epithelioma). These variations are associated with a transformation of potential into kinetic energy and indicate a considerable degree of lability of granules.

4. It is a noteworthy coincidence that this lability of cell granules, as measured by variations in the oxidase reaction, occurs in tissue with a high regenerative capacity.

5. In the nuclei, intensity of color reaction may be increased or diminished. The first occurs in degenerative processes, the latter is observed in cells which are rapidly proliferating.

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THE PEROXIDASE REACTION IN THREE CASES OF MULTIPLE MYELOMA OF THE BONES WITH REMARKS CONCERNING THE NOSOLOGICAL POSITION OF THESE TUMORS

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The exact characteristics which allow a tumor to be admitted to the myeloma group have never been uniformly agreed upon in pathological literature. From a clinical point of view the cases are fairly well defined. The presenting symptom which brings the patient to the clinic is usually pain of a constant, distressing and deep seated character, associated with marked weakness and cachexia, and occasionally with complaints leading the physician to the consideration of organic disease of the spinal cord. Physical examination shows the emaciation, loss of weight, and an anemia usually of severe grade. Careful and detailed inspection of the osseus system reveals the bone tumors usually most evident in the ribs, but often found in the long bones by the presence of pain or spontaneous fracture. The x-ray leaves no doubt of the condition, since the finding of circumscribed or diffuse bony tumors in practically all parts of the body makes the diagnosis. The Bence-Jones albumose may or may not be found in the urine. The relatively rare occurrence of this disease along with the present hopeless prognostic outlook has caused interest to be centered chiefly in the histological pathology of the tumors and the discussion of their nosological relationships.

The name multiple myeloma was first applied by v. Rustizky (1), who regarded the condition as rather a hyperplasia than a neoplasm and who believed that the characteristic cell was a marrow cell. This stand was taken because of the general resemblance both gross and microscopical which the tumor bore to

marrow tissue, rather than as a result of detailed microscopical study of the cellular elements involved. Indeed at this time the detailed histology of the marrow had not developed to a degree to make this sort of study possible.

Cases of myeloma had been described previous to v. Rustizky's account under various names, but taking the literature as a whole both preceding and following 1873, it becomes immediately evident that if many cases are to be admitted into the myeloma group we must either interpret the term liberally to mean multiple tumors in general associated with marrow, or assume the differences of description and interpretation to result from the undeveloped state of histology at that time. Similar or related clinical conditions and pathological findings are described under many names.[1]

Several important considerations relating to classification constantly arise throughout the series.

One of the earliest of these was whether the disease should be considered as a neoplasm or as an inflammatory or hyperplastic reaction of normal marrow elements. Rustizky (1) in his original description speaks of the condition as a hyperplasia rather than a heteroplasia and separates the condition from the myelogenous sarcomata of Virchow (13) on this point. Grawitz (2) reports three cases under the name osteomyelitis maligna which were probably not myelomata but aleucemic or chloromatous in nature. Abrikossoff (14) believes the cells to be of myelocyte type and discusses the position of myelomata relative to hyperplasia and neoplasia. Wieland (3) reserves the term myeloma for those new growths that are myelomatous in the anatomical sense, that is, whose structure does not depart from the mother tissue. Abrikossoff is not able to agree with Kauffman (15) and Wieland that a separation into myeloma on the one hand

[1] Myeloma multiplex (Rustizky) (1), Osteomyelitis maligna (Grawitz) (2), Lymphosarcoma multiplex ossium (Wieland) (3), Sarcoma multiplex ossium (Buch) (4), Pseudoleucaemia myelogenes (Runeberg) (5), (Zahn) (32), Ostitis sarcomatosa (Hammer) (6), Lymphadenia ossium (Nothnagel) (7), Endothelioma intravasculare (Markwald) (8), Myelosarcoma (Schmaus) (9), Erythroblastoma (Ribbert) (10), Plasmoma malignum (Hoffmann) (11), Myeloblastoma (Symmers) (12).

and multiple lymphosarcoma on the other is possible. He thinks the essential point is the inability of the myelomata to metastasize and compares them to lymphosarcomata of lymph-nodes (aleucemic leucemia?) in this respect, but separates them sharply from ordinary bone sarcomata. Since there are several undoubted myelomata now in the literature which have shown a certain degree of metastasis, Abrikossoff's basis for his conclusion is done away with. Relating to this question, it is of interest that, as time has gone on and the conception of neoplasia has become more fully developed, this matter has settled itself, and myelomata are admitted into the neoplasm family with the same doubts and reservations and the same watchful waiting regarding their ultimate disposal as is the case with the leucemias.

The main subject for settlement, however, has been the relation of these tumors to the myelocyte series of marrow cells.

The possibilities presented are obvious. These tumors might have their origin

1. From misplaced tissue not normally related to any tissue of the marrow.
2. From the ordinary connective tissue elements.
3. From the blood-vessels especially.
4. From fat tissue.
5. From lymphocytes.
6. From cells normally present and characteristic of marrow, but not part of the myelocyte series.
7. From the myelocyte series.

There is nothing in the life history or histology of multiple myelomata to suggest any such degree of heterotopia as the first consideration requires, and the possibility has never been discussed in the literature.

Before considering the remaining possibilities, the difficulty immediately arises as to what we mean by the term myeloma and how great a latitude regarding diversity of structure and power of metastasis we are going to allow to a tumor and still call it a myeloma. The disagreements between pathologists regarding the position in nosology of this tumor are always going to reduce themselves to this matter of definition until the

matter of histogenesis is settled. For the present, the author believes that a liberal conception is the best one in that the definition should contain no dogmatic statements regarding the histogenesis, and that the condition of "multiple myeloma of the bones" should be defined as a multiple primary neoplasia arising in the bone marrow, showing marked ability to erode and destroy bone with little or no tendency to reparative process or callus formation, and with very limited powers of metastasis. The histology of the tumors is characteristic and quite definite and uniform in different cases as regards the picture presented under the microscope, in spite of the uncertainty and disagreement regarding the origin of the cellular elements. On this basis we can discuss the remaining questions.

The second possibility, namely, that we are dealing with tumors of ordinary connective tissue origin, can be discarded on the ground that in the first place the cellular content of myelomata is distinctive and not that of undifferentiated connective tissue. For sarcomata of a like degree of histioid structure they are far too benign and metastasize much too sparsely. Several characters set the myeloma apart from ordinary sarcomata; their multiple primary occurrence, their distinctive and uniform cell type, with a lesser degree of heteroplasia; their specific and marked ability to erode bone by lacunar absorption without producing a reparative reaction on the part of the bone, associated with a very low degree of ability to metastasize in spite of their local destructive character.

The same considerations, together with the fact that hemangenic endotheliomata are recognized to occur (Markwald (8), Berger (16), Thevenot (17), Symmers and Vance (18)) in marrow as a distinct condition in no way resembling multiple myelomatosis pathologically, disposes of the third possibility. In order to consider the fourth division we must assume that fat is a distinct tissue of separate ancestry from connective tissue, as Mallory has suggested. Beside the fact that this idea is not generally accepted, we have the limitation of myelomata to bones rather than a relation to fat tissue in general, and the fact that while there might be a fancied resemblance between the

embryonic fat cell and the myeloma cell, the myeloma cells are more basophilic and do not differentiate like fat cells. In this connection it is of interest that Rustizky found cells in the tumors of his case that he interpreted as changed fat cells.

As to the arguments against a lymphocytic origin we are in the same position as we are with regard to fat. The myeloma tumors are always primary in bone, never[2] in lymph-nodes. They do not show even a predilection to metastasize to lymph-nodes. Moreover, multiple myelomatosis is not associated with abnormalities of the lymphatic tissue, and the tumor cells do not differentiate like lymphocytes.

On account of the fact that some cases reported as examples of myelomata have apparently been of the lymphocyte type, such as that of Herrick and Hektoen (19), there has been a tendency in the later literature to restrict the conception of myeloma definitely to those tumors having a characteristic histology. Thus Symmers objects to the cases of Herrick and Hektoen, Weber, Scheele and Herxheiner, and Kahler being included as myelomata. Symmers believes that apparent secondaries found outside the marrow usually arise by stimulation of latent centers in extramedullary hemopoietic viscera, but that true transplantation metastases do occasionally arise.

The differences in opinion as to which cases should be included as myelomata are well shown by the differences in the accepted lists of Symmers and of Pepper and Pearce (20).

The difficulty of sharply defining the limits of the myelomata has led many writers to make several groups. Thus Vance (21) distinguishes myeloblastoma, erythroblastoma, lymphocytoma, and plasmocytoma types. Christian (22), as heretofore mentioned, argues for the unity of the myeloma.

Pappenheim (23) considered them to be merely a special localization of processes belonging to the lymphosarcoma or pseudoleucemia group (Lymphosarcomatosis pseudoleucemica) and not a distinct entity in themselves.

[2] Dr. Ewing in a personal communication states that there are a few cases of (myelomata?) arising in lymph-nodes.

Sternberg (24) admits four possible groups. His first and third groups are probably identical and would now be considered as myelomata.

Some growths have been diffuse in the bone marrow rather than represented by discrete nodules. Such cases are reported by Abrikossoff (14), Weber (25), Winkler (26), Kalischer (27), and Zochmann and Schumm (28).

Paltauf (29), Sternberg, and Kaufmann differentiate the primary multiple myelogenous sarcomata and lymphosarcoma from the group of true myelomata.

These were the easily settled questions which the literature has taken care of quite adequately and which have settled themselves as the cases have been reported.

There remain, however, two additional possibilities which are most important.

(1) Do multiple myelomata constitute a group by themselves having origin from a distinct if unknown series of bone marrow cells, or—

(2) Do they belong to the myelocyte series of tumors?

These two points of view are represented by Wright (30) on the one hand, who described them as arising from "bone marrow plasma cells," and by MacCallum (31) on the other, who was satisfied as to the myeloblastic origin of his case even before the oxidase reaction came into use.

The question whether or not Wright and MacCallum were discussing different sorts of tumors is settled by Christian, who studied a series including both Wright's and MacCallum's cases and concluded that they were of the same type. Indeed from a perusal of the typical cases reported in the literature, such as those of MacCallum, Christian, Wright, Vance, and Symmers, and even the older cases of Rustizky, Zahn (32), and Wieland, there can be little doubt that the multiple myelomata represent a distinct tumor type possessing distinctive histological features and differing from sarcomata in general and from the leucemic group. There are no greater differences in cell type in members of individual cases than between any other well defined tumor group. This idea has been especially developed by Christian,

who showed that there were not sufficient differences between Wright's and MacCallum's cases to warrant placing them in separate groups, in spite of the fact that the opinion of these two workers differed as to the resemblance of the cells to plasma cells on the one hand and myeloblasts on the other. Christian leaned toward the view that they resemble plasma cells more than myelocytes.

The oxidase reaction has added to our methods for the investigation of these tumors in that it may be applied now to each new case encountered. It must be clearly kept in mind, as Symmers pointed out, that a negative reaction does not prove that the tumor is not of myeloblastic origin. More than this, the author does not find proof in the literature that other cells of the marrow than the myelocyte series *may* not act positively to the oxidase reaction. It must be remembered that the lymphocyte series have been found to react negatively and the more differentiated members of the myelocyte series to act positively under normal conditions; and that while it is logical on these grounds to resort to this technique for deciding the origin of a circulating blood cell, it is quite another and unjustified leap of logic to assume that in the bone-marrow no cells but the myelocyte series give the peroxidase reaction even under pathological conditions. The point is that while the latter assumption may be true the question has not been studied.

In other words the peroxidase reaction applied to myelomata is productive of interesting information, but until we have more definite knowledge of bone-marrow histology nothing is actually settled by applying this reaction to a particular case. Furthermore there are other important general considerations which point toward a solution without the help of the oxidase reaction. To the writer's mind a very important point of view is that of Mallory (33), that these tumors do not consist of cells of the myelocyte series because they do not differentiate like them.

Since Wright claimed a "bone-marrow plasma cell" origin for myelomata there have been many reports either endeavoring to prove or taking it for granted that the tumor arises from the myeloblast series. The objection that has been raised to Wright's

view has been that the bone-marrow plasma cell is not a clearly defined entity. As Ewing (34) says, the histological structure of some of the cases so closely resembles plasma cells as to suggest that those who deny their existence have not encountered these cases. The oxidase reaction has revealed a few cases which have reacted positively and which, if we accept this reaction as positive proof of a myeloblastic origin, might be interpreted as myeloblastomata. Forman and Warren (35) report such a case and also Beck and McCleary (36). Both of Symmers' cases were negative as were also those of Pepper and Pearce, Bombard (37), and Vance along with three included in this report.

Aside from the occasional positive peroxidase reaction, the dictum that myeloma multiplex arises from the myeloblast series rests entirely upon an effort to show that the tumor cells resemble myeloblasts morphologically.

This is altogether insufficient evidence considering the fact that its champions object to assigning the tumors a plasma cell origin, although admitting a similar resemblance morphologically. If a resemblance to plasma cells is not a sufficient proof of plasma cell relationships, then a myeloblastic resemblance does not prove myeloblast relationships. Taking the literature as a whole we find both ideas with ample supporters. Thus Von Verebely (38), Thomas (39), Vance, Wright, Christian, Hoffmann (40), Aschoff (41), Pepper and Pearce, and Ewing recognize plasma cell types, while MacLeod (42) and Zininger (43) note a resemblance to plasma cells. Menne (44), MacCallum, Abrikossoff, Weber (45) (Muir), Ribbert (46), Scheele and Herxheimer (47), Sternberg, Jores (48), Symmers, Forman and Warren, Beck and McCleary, believe in the myeloblastic origin.

The question cannot be settled on morphological grounds because of the limitations of the method.

There are several difficulties in the way of considering myelomata as myeloblastic tumors.

In the first place, if the cells of myelomata are myeloblasts or premyeloblasts and belong to the myelocyte series, why are these tumors not chloromata and why do we not find transition stages in structure between myelomata and chloromata? The fact is

that myeloma cases never become chloromatous and never become associated with true leucemias, as do all chloromas sooner or later. Furthermore, myeloma cases do not exhibit the changes in the spleen and lymph-nodes that chloroma cases do. It might be argued that the myeloma cell is a less differentiated member of the hemopoietic group and therefore does not possess the highly differential morphological characters of the chloroma cell. Against this is the fact that the chloroma is more malignant than the myeloma, both as regards rapidity of growth, metastasis, and a rapidly fatal issue; which does not argue well for the chloroma possessing a more highly differentiated cell.

In short, chloromata belong to the myeloblast series, are rapidly fatal, and always are, or become, associated with leucemias; myelomata do not differentiate like myelocytes and never become leucemic. Chloromata metastasize widely and are associated with characteristic leucemic marrow and with lymph-node and splenic changes; myelomata do none of these things.

If it were not for the definitely myelocytic or lymphocytic character of chloromata and the definite leucemic nature of these tumors, the possession of a green color alone would not be sufficient to separate them from the myelomata. *To the writer's mind, this failure of myelomata to differentiate like myeloblasts, and the absence of a relation between myelomata and leucemic states are obstacles to accepting myelomata as myeloblastic tumors, no matter how strong morphological evidence based on cytological comparisons may appear to be.*

One most distinctive character possessed by the myeloma is its ability to erode and destroy bone without a reaction being set up. This is striking throughout the literature if we disregard a few isolated cases like the atypical one of Hammers (49), which was was probably not a myeloma. The bone absorption goes on steadily and extensively without attempt at repair, and is certainly a specific property of the tumor cell. Neither normal nor neoplastic cells of the myeloblastic series have this property and the chloromata, although much more malignant than the myelomata, possess very little bone destroying power and the ordinary leucemias scarcely any. The albumosuria is probably

associated in some way with the bone destroying power since other destructive bone lesions such as metastatic carcinomatosis sometimes show it and the chloromata and other forms of leucemia only rarely. So far this is in support of the general view Ewing expresses regarding the different identity of typical myelomata and tumors of the leucemic (myeloblastic) group.

A point of view hitherto undiscussed in the literature is that perhaps myelomata belong to that series of reticulum cells whose function it is to absorb bone and regulate bone formation. The statements in the literature regarding bone absorption are unsatisfactory. Two sorts of cells having to do with bone formation have been described, osteoblasts and osteoclasts. By some these have been considered as the same and by others as representing distinct types. The classical cell to which osteoclastic function has been ascribed is a multinucleate giant cell of the foreign-body type. These cells are numerous in areas where large amounts of bone are to be removed such as in healing fractures, but it is remarkable how scarce they are in many sorts of bone formation where a very evident bone resorption is going on in the lacunae.

There has never been sufficient proof that the multinucleated giant cell, which since the work of Kölliker has been assumed to be the active agent in bone absorption, has in fact this function. A very casual survey of sections of actively growing bone, showing evident resorption around the marrow spaces, makes it evident that much bone absorption must go on without the intervention of the classical osteoclast.

This has been recognized by many authors. Arey (50) in a recent important article gives the literature and shows definitely that many workers have been of the opinion that classical osteoclasts are not important in ordinary bone resorption. He concludes that "Only indirect and insufficient evidence points to the osteoclasts as the active specific agents of bone resorption. That they are merely degenerating, fused osteoblasts accords better with known facts." This leaves us with the alternative that the so-called osteoblast must in some way regulate bone absorption as well as bone deposition. This view is favored by

Arey, who believes that bone absorption is brought about as Wells thought by CO_2, although he recognizes that it is "difficult to imagine the mechanism of the localization of carbon dioxide (or the stronger lactic acid) in sufficient concentrations to effect the selective erosion of small areas or to account for the frequent directional polarization of the resorptive wave."

Studying smears and teased preparations from myelomata, the author has been very much impressed by the resemblance

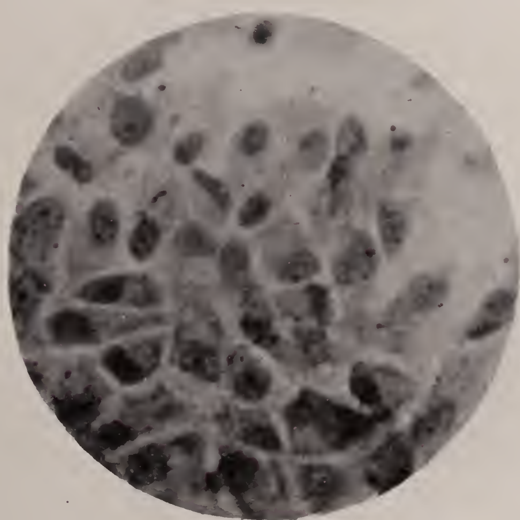

Fig. 1. Osteoblasts in an Osteoma

which the cells from the tumors bore to the osteoblasts(?) which lie free in the connective tissue of the marrow and especially line the lacunae of rapidly growing bone. This is exemplified by figure 1, which is a photograph of a group of osteoblasts on the tip of a young bone trabecula in an area from the marrow of a rapidly growing ungual osteoma. The basophilic protoplasm, the eccentric nucleus of the vesicular type with peripherally arranged chromatin and central nucleolus are especially striking.

It is easy by measurements, staining reaction, and description of cytoplasmic and nuclear structure to show the similar appearance of the "osteoblast" and myeloma cell, but to use this method to establish the identity of the two cells is unjustifiable here as in the case of those who on similar grounds claim to show that myeloma cells are myeloblasts.

The important property of bone absorption which the myeloma cell so prominently possesses, accords well with an inferred relationship to cells of the osteoblastic series. On the grounds of the preservation of this function, we might infer a relative lack of anaplasia and consequent lack of malignancy which again accords with the facts. Only one difficulty arises. If these cells are osteoblastic in their relationships, why do we always find these tumors actively resorbing bone, but never building it?

Either of two assumptions might be favored. First that the cells of the myeloma differentiate in this special direction, and by this mode of differentiation bone absorption is made a hyperfunction just as the production of colloid material by some tumors arising from cells which normally produce it becomes an essential characteristic of their heteroplasia. This requires the conception of a constantly persistent "resorptive wave" to use the euphonic but somewhat vague term of Arey.

The second alternative we might use is to question the fundamental correctness of the idea that the so-called osteoblast is really a bone formative cell and suggest that it is always resorptive in function. This assumption is against the present conception of osteogenesis.

The clinical records of the three following cases have been left out for the reason that they contain no details bearing on the present study.

Case 1. *Autopsy one hour after death.*—The body is that of a very much emaciated man 37 years of age. A globular mass mentioned and described in the clinical history is found involving the left shoulder and clavicle. The seventh rib on the left is fractured in several places and is soft, easily broken, and paper-like throughout. Fractures can also be palpated in the fourth and fifth ribs on both sides.

On section the muscles are dark, dry, and small; the panniculus is absent. Superficial examination of the abdomen reveals the viscera in proper position and no fluid or exudate in the abdominal cavity. On removing the sternum we find it to be thicker than normal and soft and friable, so that large soft portions can be cut out with the knife. By making a mesial section and dividing the sternum in two portions the bone cortex is found to be a mere shell, largely decalcified, and the marrow to occupy almost the entire thickness of the sternum (about 4 cm.). The marrow is mottled pink in color and resembles the cut surface of a lymph-node. There are small hemorrhages and islands of bone fragments. Scarcely any areas of normal red marrow remain.

The ribs are thin-shelled, nearly decalcified, and there are nodules of tumor bulging and breaking through the bony cortex. Elsewhere the medulla of the ribs consists of dry, empty spaces. The right and left lungs are both adherent at the apex and present a low grade, nearly healed tuberculosis. There is anthracosis of the bronchial lymph-nodes.

The heart is of normal size. On section the muscle is deep brown. The aorta presents a moderate nodular sclerosis, evidenced by raised yellowish plaques. In the muscle of the ear of the right auricle, beneath the epicardium, is found a pinkish white plaque similar in appearance to the tumors found in the ribs and sternum. This plaque is about the size of a pea. The heart is otherwise normal.

There is no evidence of thymic remains and the thyroid is of the usual size and on section is found the usual colloid appearance without adenomata.

The gastro-intestinal tract including the liver and pancreas was examined in detail without any important finding. The liver is browner than normal, and appears slightly atrophic (brown atrophy).

The abdominal aorta is moderately sclerotic like the thoracic portion.

The spleen is of the usual size and consistency, and on section seems normal in consistency and appearance.

Both kidneys present numerous white nodules in the cortex. These vary in size from a pin-head to a hickory nut, and have a pinkish white color and lymphoid appearance. They are identical with the tumors found in the ribs and the sternum.

The prostate and bladder are found to be without unusual appearance.

The vertebrae can be freely incised with the autopsy knife. They have almost entirely lost their lime salts and are replaced by the lymphoid-like soft material heretofore described.

Microscopical findings.—The tumor nodules (fig. 2).

The sections of the tumor taken from all the different bones present the same picture. The structure is purely histioid consisting of a uniform mass of closely packed cells with a very delicate reticulum. The blood vessels are without walls, and are in the form of endothelium-lined spaces running through the tumor. All fields present the same picture. There are no areas of degeneration, necrosis, or fibrosis.

At first glance the cell type seems remarkably uniform. The cells are irregularly oval and fairly uniform in size with most of the nuclei eccentrically placed. The protoplasm is slightly basophilic and stains like the protoplasm of plasma cells, and the margins of the cells when not pressed on all sides by other cells tend to be somewhat ragged. On closer analysis the individual cells are found to vary from one another to a considerable degree, but no more widely than those of any tumor which is rapidly growing. The cells vary from an oval shape like that of the plasma cell, to an irregularly round or polygonal outline in those areas where they are pressed upon by other cells. They vary in size from 5.8 to 8 microns in the short diameter, and from 7.7 to 13 microns in the long. The nuclei are more variable in size than the cells themselves. Some of them are small and pyknotic and take up only one end of the cell and others occupy nearly all the cell body. They vary in size from 5.8 x 9.1 microns to round pyknotic forms measuring 3.6 microns. In some of the cells the nuclei have divided without cell division following, so that cells resembling bone marrow megakaryocytes are produced.

Though they vary somewhat in size and shape, the great majority of the nuclei are vesicular in character with definite nuclear membranes which take the stain deeply. The chromatin is rather small in amount, giving the nucleus the appearance of a clear granular vesicle; it is for the most part murally arranged, clinging to the inner side of the nuclear membrane with delicate spiderweb lines running centrally to the nucleolus, which is

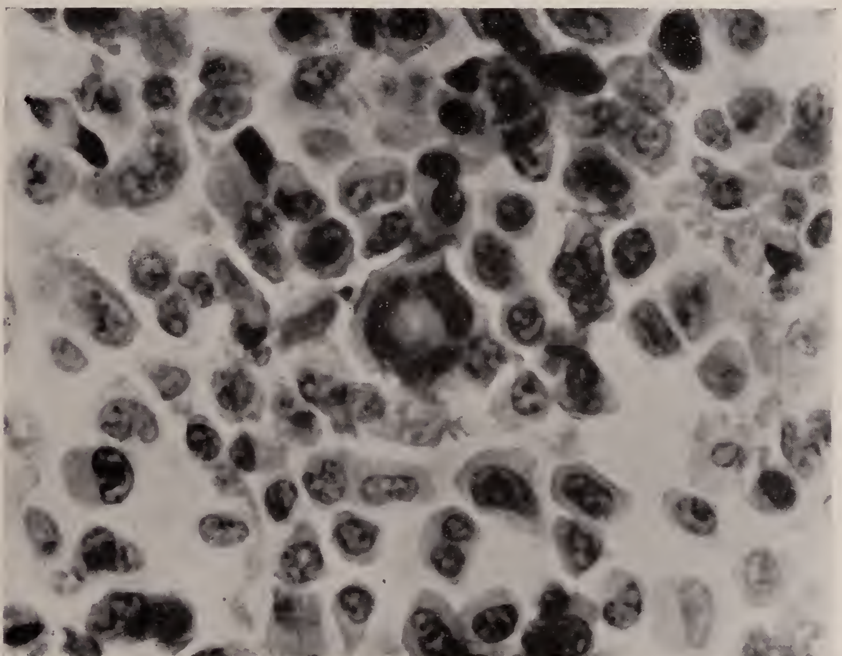

Fig. 2. Cells of Myeloma, Case I

usually prominent, and varies in size from a tiny chromatin point to a mass measuring about 1.8 microns in diameter. Occasionally there appear to be two nucleoli.

The sections of tumor taken from the body of the vertebrae show the contiguous muscle to be diffusely invaded. In places bony trabeculae are still present, but undergoing absorption. There are no osteoclasts, but the tumor cells are present in the lacunae and canaliculae and appear to be performing the osteo-

elastic function. There is no effort at bone formation in any of the sections.

The peroxidase reaction applied by both methods, to smears and frozen sections, was negative.

Kidney.—There is no diffuse infiltration of the kidney parenchyma with the tumor, but there are discrete nodules beneath the capsule about the size of a pea. These nodules have the same histological characters as the primary tumors. Remains of kidney tubules are scattered through it, and glomeruli with their tufts containing many fatty droplets. The uninvolved portion of the kidney shows many areas where the tubular epithelium is completely calcified but no other important changes. An occasional thrombosed glomerular tuft with the capsule filled with wandering cells and an occasional hyaline glomerulus appears, but there are no diffuse changes which could be interpreted as a definite nephropathic condition.

Lung.—There is an advanced emphysema, with the usual accompanying histological changes and a diffuse anthracosis. There are a few heart failure cells. Some of the branches of the pulmonary artery show quite marked proliferation of the intima. The lung tissue as a whole is much more fibrous than is usual in even the more advanced types of emphysema.

The liver presents no important changes. The cells around the central vein contain a great deal of light brown pigment and there is some pressure atrophy of the liver cords in this region with many fat droplets in the cells (early nutmeg liver).

Gastro-intestinal tract negative.

In the heart muscle there is no change except many fine fatty droplets in muscle cells, and increased brown pigment at the poles of the nuclei.

Thyroid.—Moderate enlargement of the follicles. Apparent colloid retention.

Spleen.—The pulp is especially prominent. The Malpighian bodies are few in number and atrophic. The pulp appears remarkably cellular and there are wide sinuses running through it. There is no increase of connective tissue in the spleen pulp.

Bone-marrow.—In areas not grossly involved by tumor growth the normal marrow elements are replaced in part by tumor cells, interspersed in large numbers.

Pathological Diagnosis.—Multiple myeloma of the bones, metastasis to the kidneys and muscle of right auricle. Metastatic calcification of the kidney tubules.

Case 2. *Autopsy.*—Body is that of a man 35 or 40 years of age. Well formed, distinctly emaciated. Faint icteric tint to the sclera. Moderate purplish hypostasis on the back.

Palpation of the bony cage of the thorax shows many softened crepitating areas on both sides. The left femur just above the knee is fractured when the body is moved from the stretcher to the morgue table. There are no other external abnormalities of importance.

On section the tissue is dry, the muscles dark red. No gas or fluid escapes from the abdominal cavity. Superficial inspection of the abdomen shows no gross abnormality. There is a moderate pseudo-melanosis of the intestines. The fifth rib is softened at the costo-cartilagenous junction, crepitates, and feels irregular on palpation.

Dissecting the tissue away from this region, we find an opalescent bluish mass the size of a hickory nut, and from here to the fourth rib at the costo-cartilagenous junction the tumor mass extends. The tumor tissue found on the fourth and fifth ribs replacing the bony cortex, resembles lymphoid tissue in general. On taking off the lateral wall of the chest with rib shears we find the ribs involved throughout their length by nodules of lymphoid-like tissue, which shine through the pleura presenting a linear nodular appearance along each rib. The ribs are extremely fragile, containing very little lime salts. The tumor mass, primary in the ribs, has completely replaced the rib marrow, everywhere, and in certain places has broken through the cortex and appears as an external nodule.

Removing the sternum with the attached costal cartilages, we find it very flexible, containing very little bone, and much thickened at the lower half. The sternum was split longitudinally with the autopsy knife. Scarcely any lime salts were present.

The marrow has been replaced by a lymphoid mass. The whole costal cage is replaced by lymphoid-like nodular tissue.

The apex of the heart is at the fourth interspace, apparently 1 cm. outside the nipple line. The right heart extends beyond the sternum. Both lungs are free and have collapsed about the hilus. The heart has a small soldier's spot on the right ventricle. The left ventricle is empty. The wall of the left ventricle is normal in thickness and color. The mitral valves admit two fingers. The flaps are normal. The right ventricle is empty. The tricuspid is relatively dilated. The right auricle contains a large post-mortem clot. The pulmonary artery presents no abnormality. The aorta shows a fine nodular type of sclerosis, not particularly advanced. The left coronary mouth is greatly dilated. There are patches of atheroma along the left coronary and also along branches of the right coronary. No gross evidence of syphilis is present in the heart. There is a patch of old adhesions on the posterior surface of the left lung near the vertebrae. The adhesion seems continuous with the tumor in the rib. The tumor has slightly involved the pleura at this point. The lungs are small and dark in color, and on section fairly normal. Mediastinal lymph-nodes show no involvement. There is a purulent discharge from the bronchi. The right lung is small with a few subpleural nodules in the upper lobe. These nodules are calcified, the size of a pea, probably a healed tuberculosis. The right lung is otherwise negative.

The thoracic aorta is normal except for a slight nodular sclerosis.

The spleen is normal in size. There is considerable post-mortem autolysis and on section a distinct hyperplasia of the lymphoid tissue. The left suprarenal is normal in size, and on section shows no changes.

The left ureter is not dilated. The left kidney is small and has a scanty fatty capsule. On section the cortex is pale and swollen. Medullary rays are not well marked. The capsule strips easily.

The right adrenal is normal in size, and on section negative. The right kidney is about normal in size, and on section presents the same appearances as the left.

The mesenteric lymph-nodes are not enlarged. The intestines contain many fecal masses, but are otherwise negative.

Pancreas presents no abnormal gross changes.

There is a moderate purulent cystitis.

The prostate is normal in size.

The liver is of normal size and on section presents a slightly browner appearance than normal.

The gall bladder is distended, but contains no stones.

Pathological diagnosis.—Multiple myeloma of the bones, involving nearly all the bones of the body.

The x-ray report of a mass in the mediastinum was based upon an appearance due to the wide infiltration of the vertebrae in the thoracic region by the tumor. On either side of the vertebrae in the upper thorax the infiltration extends outward along the transverse processes and the proximal portion of the ribs in such a manner as to produce a large pyramidal-shaped x-ray shadow, which might very well be mistaken for a mediastinal growth.

The following is a summary of the histological changes.

The heart, lung, pancreas, bladder and adrenal present no changes of importance.

The spleen shows a distinct hypertrophy of the lymphoid follicles. There are no important changes in the pulp. No cells resembling tumor cells are found in the pulp. There are a few miliary tubercles in a moderate stage of fibrosis.

There is no increase in connective tissue in the kidney. Glomeruli appear normal. The cells of the tubules are granular from postmortem change. Throughout the cortex are many tubules which have been replaced by lime salts.

Pieces were taken from the tumor nodules of almost all the bones in the body, for microscopical examination.

The picture presented is uniform throughout (fig. 3). The tumor consists of a uniform histioid structure with a uniform type cell arranged on a delicate reticulum, with an intimate relation between the tumor cells and the blood-vessels. The blood-vessels have no proper wall, but pursue their course through the tumor lined by a simple layer of endothelial cells. The tumor

cells have the usual resemblance to plasma cells. They are of the same size and variation as in the previous case. In general they have eccentrically placed nuclei, basophilic protoplasm, with large vesicular nucleus showing the characteristic mural arrangement of chromatin with fine threads projecting inward toward the nucleolus. Although the ribs, vertebrae, sternum, long bones, and bones of the pelvis were involved in this case,

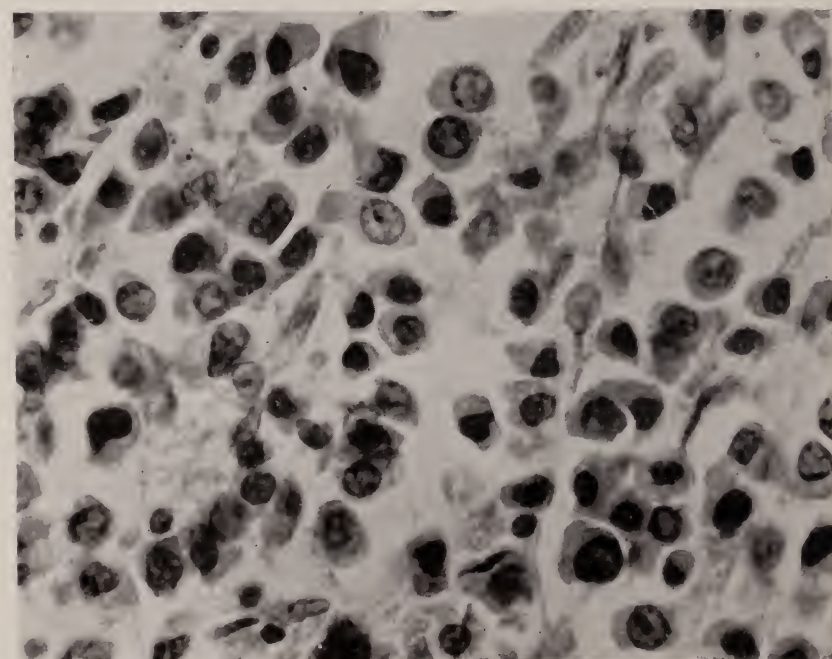

Fig. 3. Cells of Myeloma, Case II

there were no metastases to other organs as in the previous case. The metastatic calcification of the kidney was quite as striking as in case 1. There were no other pathological changes of importance found by microscopical examination of the sections.

The peroxidase reaction applied to smears of the tumor as well as to frozen sections of tissue fixed in formalin were negative by both methods.

CASE 3. *Autopsy findings.*—The body is that of an adult male, about 40 years of age, 5 feet 11 inches tall. Greatly emaciated. The bony landmarks are normal throughout.

About 10 cm. to the right of the mid-sternal line on the third rib is a subcutaneous lump the size of a hen's egg and a smaller lump in the same region in the first rib under the clavicle. These lumps are connected with the rib and continuous with it.

There is some enlargement of the left testis, which is soft and elastic.

The abdomen is slightly distended. On section the panniculus is found practically absent. Tissues are moist and the muscles dark red. Superficial inspection of the abdomen shows a small amount of fluid in the abdominal cavity, but the gross relation of the organs is normal.

On stripping back the muscles from the thoracic cage we see the nodules above described developing from the ribs. The bony structure of the ribs is soft and friable. The mass is enlarged, has broken through the bony cortex of the ribs, and on section one obtains a thick semi-gelatinous substance, somewhat resembling pus.

The right lung is adherent in the upper portion and laterally by well organized fibrous tissue.

The left lung is free, but throughout there is considerable hypostatic congestion.

The right lung shows marked congestion, some edema. There are no solid areas of pneumonia and the bronchial lymph-nodes are not enlarged. There is considerable anthracosis. The left lung is less congested than the right. Both lungs crepitated throughout.

The heart lies free in the pericardial sac. There are no adhesions. There is a small amount of free fluid in the pericardial cavity. The mitral valves admit the tips of two fingers. There is marked sclerosis of the mitral cusps. The tricuspid valves are negative. The right orifice is empty, the left oiifice admits two fingers, but there is no evidence of sclerosis. There is considerable edema of the walls of the coronary arteries. The aortic semilunars are slightly sclerotic. The first portion of the aorta

is somewhat dilated. The intima has a slight reddish discoloration, from a post-mortem imbibition of hemoglobin. There are no other changes of importance in the thorax.

In the abdomen there is about 200 to 500 cc. of clear fluid. The relation of the abdominal viscera is not disturbed. The liver is slightly enlarged, extending about two finger-breadths below the costal margin. It is smooth, the capsule somewhat thickened. On section the liver bleeds easily, and the centers of the lobules are dark red in color. The spleen is normal in size; the capsule somewhat thickened. On section there are no important gross changes. On removing the intestines we find the pyloric end of the stomach adherent by a mass of soft friable tissue. The mass involves the wall and the pyloric ring, and on section has the same general character as the mass found in the rib. This mass is continuous with the posterior wall of the abdominal cavity where it extends into the 8th and 9th dorsal vertebrae. These vertebrae are completely destroyed by the tumor, and the mass in the stomach and the abdomen is evidently a direct extension from this point.

Inspection of the vertebral bodies as a whole shows practically the whole spine involved in the process, with some areas of fairly normal vertebral tissue between the nodular masses of tumor substance.

The retroperitoneal lymph-nodes are somewhat enlarged.

The pancreas is soft, slightly displaced by the mass adherent to the pylorus.

The kidneys are somewhat congested, but show no other gross changes.

The prostate is slightly enlarged.

There is a large right-sided hydrocele of the testis, with some atrophy of the organ. The left testicle is slightly enlarged, but shows no gross changes.

Pathological diagnosis.—Multiple myeloma of the bones. Direct extension to the stomach. Hydrocele of the right testis.

Microscopical findings.—The lung shows a distinct emphysema with areas of atelectasis. There is fibrosis of the interalveolar septa, and many heart failure cells in the alveoli. The

bronchi are filled with a thin mucous secretion. There is an advanced anthracosis. There is a considerable hyperplasia of both the spleen pulp and the Malpighian bodies. The spleen pulp shows an increase in its cellular content, and under high power many of these cells resemble those of the tumor. In the margins of the Malpighian bodies are also large numbers of large mononuclear cells, identical morphologically with those found in the tumor nodules. There is considerable increase of connective tissue in the pulp. The kidney presents an appearance of post-mortem disintegration of the protoplasm of the tubules. No changes are found which can be interpreted as nephropathic.

In sections of the prostate there are found no inflammatory areas or areas of glandular hyperplasia.

There are no changes of note in the heart muscle.

Sections were made from the tumor nodules of the rib of the involved vertebrae, and of the stomach region involved by continuity with the vertebral tumors. The tumor is of a pure histioid character, consisting of round cells arranged on a delicate connective tissue reticulum (fig. 4). There are some larger bands of hyaline connective tissue running through the tumor. The blood-vessels have delicate walls consisting of a few connective tissue strands and lined by a delicate endothelium. The cells in general closely resemble plasma cells. They are somewhat ovoid in shape with an eccentrically placed nucleus and a basophilic protoplasm; their margins tend to be somewhat ragged where the cell is not pressed upon from all sides. The nucleus is vesicular with murally arranged chromatin and fine bands of chromatin running toward the center to a rather large nucleolus. The structure is typical of a myeloma of the so-called plasma celled type.

In none of these three cases were abnormal cells observed in the peripheral blood. The many cases in the literature in which myelocytes in small numbers have appeared along with a terminal polymorphonuclear leucocytosis are explained on the ground that such terminal blood pictures are common with advanced tumors, especially when the bone-marrow is involved. Our cases show the terminal leucocytosis common to late malignancies, as pointed out by Vaughan (53).

The author does not find convincing statements in the literature that "plasma cells" or cells of distinctive type may be found during life in the circulating blood and used for diagnosis. The case of Beck and McCleary is certainly open to question regarding the interpretation of the origin of the cells found at autopsy in the blood-vessels. In the first place these cells had already been interpreted as ordinary blood cells during life, and not

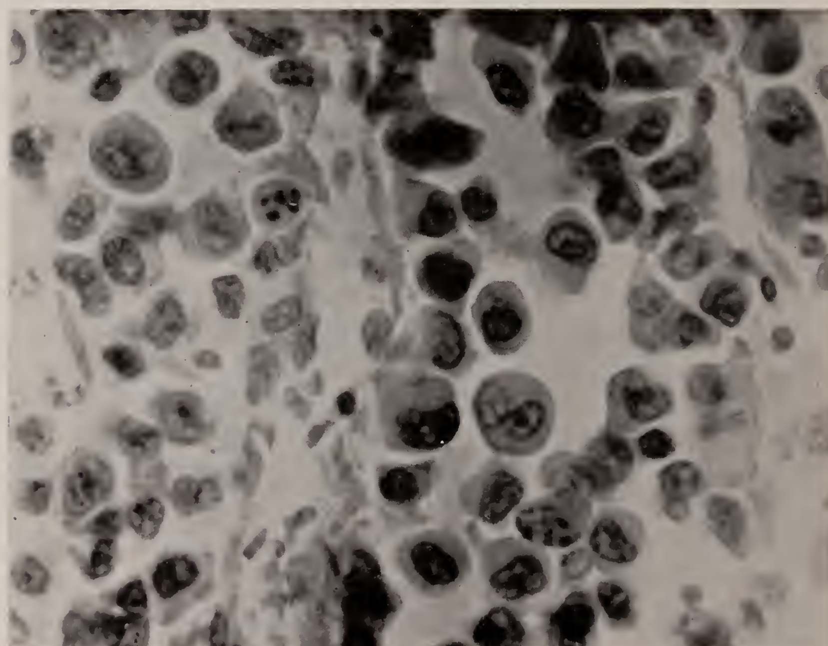

FIG. 4. MYELOMA OF THE "PLASMA-CELL TYPE," CASE III

until after death was it decided that they represented distinct tumor cells. Further, although the patient is said to have died of acute lobar pneumonia, the white blood count is recorded as 8800. The tumor cells in their case gave the indophenol blue synthesis, but they do not record its result with blood smears.

The finding of abnormal cells in these cases must be interpreted as a result of combined reaction to late malignant disease in general and to non-specific bone marrow involvement in particular.

The peroxidase reaction, both on frozen section and on smears from the tumor surface, was negative by both methods tried. The methods in detail are given below.

THE PEROXIDASE REACTION

Two methods were used on both smears and frozen sections. The technique used by Forman and Warren was applied, which is similar to the one used by Evans (51) except that some steps are omitted.

The frozen sections from formalin-fixed tissue were placed in equal parts of 1 per cent alpha-naphthol in 1 per cent KOH and of 1 per cent dimethylparaphenylendiamin for two minutes. The section was then washed and examined in water on a slide with a coverglass. Smears were fixed in formol-alcohol for two minutes, washed, and the same technique applied.

The second method used was that of Goodpasture (52). The stain is prepared as follows:

Alcohol 95 per cent	100.0 cc.
Sodium nitroprusside	0.05 gm.
Benzidine (C. P.)	0.05 gm.
Basic fuchsin	0.05 gm.
Hydrogen peroxide	0.5 cc.

The sodium nitroprusside is dissolved in as little water as possible and then added to the alcohol; the other ingredients are then included. Goodpasture's solutions retained their activity for several weeks. I have not been able to get the reaction except with freshly prepared solutions.

Smears are allowed to dry and then covered with the reagent for one minute; an equal quantity of water is added and allowed to remain three minutes when the preparation is washed, blotted, dried, and mounted in balsam.

Frozen sections of formalin-fixed tissue are placed in equal parts of the reagent and water for five minutes, washed in water, dehydrated in acetone, cleared in xylol, and mounted in balsam.

The oxidase granules are blue, the nuclei red, and the protoplasm pink.

Blood smears and frozen sections from an inflamed Fallopian tube and appendix were used to control the stain.

In all three cases only a few scattered leucocytes showed the characteristic granules in either sections or smears. From this result we can only conclude that if the cells are of the myelocyte series they are too imperfectly differentiated to give the reaction.

SUMMARY AND CONCLUSION

1. Three cases of non-oxidase reacting myelomata are reported.
2. The histogenesis of these tumors is discussed, and data presented which is interpreted to support the theory that the so-called "plasma cell" type of myelomata is not of myeloblastic origin and has no relation to the leucemic group.
3. It is suggested that the "plasma cell" myelomata spring from a series of cells whose specific function is bone absorption, and that the myeloma cell may be a heteroplastic "osteoblast."
4. The finding of abnormal cell types in the peripheral blood of myeloma cases has not been demonstrated by the published examples to be specific and characteristic of this form of tumor. The myelocytes, "plasma cells," and other abnormal cell types, together with varying degrees of leucocytosis and disturbances of the percentage relationships of the various normal leucocytes, are adequately accounted for by the condition of malignancy accompanied by wide-spread bone-marrow involvement, and is found in non-myelomatous conditions.

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THE INFLUENCE OF CERTAIN DIETS UPON TUMOR SUSCEPTIBILITY AND GROWTH IN ALBINO RATS

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The question of the relationship between neoplastic growth and the food supply of the host has received the attention of a number of investigators. Such studies have not been limited to experimental animals, and a number of writers have suggested a relationship between diet and malignant disease in man. Thus Ross (1) reports that among the natives of the Arctic region cancer is rare. It has been suggested that the rarity of cancer among the natives of Africa, India, the East Indian Islands, China, Japan, and Brazil, is due to their living entirely upon a vegetable diet. However, those who have adopted the European life and eaten a large quantity of meat are not exempt from malignant disease (2–6). On the other hand, Hendley (7), Bentall (8), and Bashford (9) have reported that the occurrence of cancer among the vegetarian Japanese and Indians is as great as among those indulging in a mixed diet.

It may be of interest in conjunction with the present paper to review briefly what dietetic studies have been made on the nature and cause of malignant growth.

Haaland (10) has shown that mice fed upon a diet consisting of hemp-seed, bread, milk, and oats are less resistant to a transplantable Ehrlich mouse sarcoma than those fed upon a less complex diet of bread and oats.

Stahr (11) has reported that a diet of hemp-seed and milk makes mice less susceptible to tumor inoculation than those fed upon bread and water.

Moreschi (12) studied the development of tumor grafts in underfed mice, and found that these growths are very much retarded by such treatment. His results were confirmed by Rous (13).

Sweet, Corson-White, and Saxon (14) showed that the number of takes of a transplantable mouse carcinoma is greatly reduced and the rate of tumor growth is retarded if the hosts are fed upon Osborne-Mendel's artificial diet (15) in which certain amino-acids are lacking. A similar experiment with Flexner-Jobling rat carcinoma showed the same results and they drew the conclusion that tumor cells and normal somatic cells are subject to the same laws of growth (16).

Van Alstyne and Beebe (17) found that if the hosts were fed with non-carbohydrate diet from two to six weeks previous to transplanting tumor tissue, the resistance of the animals to the tumor became greater and the growth of the grafts was retarded as compared with those fed upon a complete diet. They recalled the work of Robertson and Burnett (18) and suggested that the difference in tumor susceptibility in animals fed upon a mixed diet of oats and meat and exclusively milk diet is partly, if not entirely, due to quantity difference in the carbohydrate content of the diets.

Rous (19) made an extensive study of the influence of Sweet's modification of Osborne-Mendel's artificial diet on tumor grafts and on spontaneous tumors. He showed that such diet had no specific influence upon the growth of Flexner-Jobling adenocarcinoma, but had a distinct retarding action upon the metastasizing mouse carcinomata. In studying spontaneous tumors he obtained the following results: first, that underfeeding the tumor-bearing animals with such diet for some days prior to the removal of all except a small portion of tumor delayed recurrences and the growth of tumor grafts; second, changing to the special diet from normal diet after the operation had no marked effect on the number of recurrences or the time required for their appearance, though the growth of grafts was somewhat retarded; and thirdly, that unoperated spontaneous tumors appeared to be unaffected by the most drastic dieting. From these results he

concluded that the dietetic influence upon tumor growth may be "attributed solely to the underfeeding and resultant loss of weight, and not to the character of the food" (20).

Funk (21) reported that chickens (Plymouth Rocks) fed upon polished rice a few days prior to inoculation of Rous's chicken sarcoma showed no takes; however, if they were fed upon unpolished rice or polished rice with the addition of a small amount of yeast, the tumors developed. A similar experiment by Levin (22) did not support Funk's observations.

One of the present writers and Rahe (23) found that an artificial diet free from water-soluble vitamines had a retarding action upon the development of tumor grafts, and that the tumors could obtain a supply of necessary vitamines from the tissues of the hosts.

Drummond (24) has studied the influence of the following dietary inadequacies upon tumor and normal tissue growth: (a) low protein content of the diet; (b) nitrogen of the diet supplied in the form of a protein possessing a relatively low nutritive value; (c) absence of certain indispensable amino-acids from the diet; and (d) absence of the equally indispensable accessory growth promoting factors, the "fat-soluble A" and the "water-soluble B." The artificial diet proposed by Osborne and Mendel (25) was used. Drummond's experimental results corroborate the findings of Sweet and associates (16) as to the amino-acids deficiency; of Benedict and Rahe (23) as to the "water-soluble B" deficiency; and of Rous (19) as to the undernourishment of the hosts, and he has lastly stated "that only the most drastic restrictions, involving a very serious loss of weight upon the part of the host, have any retarding influence upon tumor growth."

In the present study we have selected the banana as the basis of our experimental ration. It is the main article of diet of the natives of several tropical countries (Egypt, Brazil, etc.), where the incidence of cancer is low. The nutritive value of the banana has already been studied (26). The fruit is deficient in (a) protein, and (b) certain food accessory factors or so-called *vitamines*.

EXPERIMENTAL

Methods and materials employed

Throughout the present experiments we have used the Flexner-Jobling rat carcinoma which was kindly furnished by Dr. Wood,[1] Director of the Crocker Laboratory of Columbia University. This tumor was in the 60th generation at the time we obtained it, October 22, 1918, and was designated in that institute as $\frac{\text{FRC}}{\text{60D}}$.

The previous history regarding this specific tumor has been given by Flexner, Jobling, and others. The tumor was very malignant in character, having, (a) power of uniform, rapid, and continuous growth; (b) power to metastasize, etc.

The tumors to be used for the successive transplantations were selected among rapidly growing tumors which had not ulcerated. The age of the tumors was about four weeks.

The general routine of the tumor inoculations (27) was as follows: After killing the tumor-bearing animal with ether it was submerged in 0.1 per cent of mercuric chloride or 1 per cent of lysol for about a minute, and then in 95 per cent ethyl alcohol for about the same time. The tumor was removed by sterile instruments and transferred to a sterile Petri dish. A portion of the tumor was fixed in a formaldehyde solution for histological examination. The subcutaneous inoculation of the tumor fragments into new hosts was done by a trochar which was sterilized by alcohol and heat and cooled in sterile water between inoculations. Tumor tissue weighing about 10 mgm. was selected from the non-necrotic area of the tumor and was inoculated into the region of the right axilla. The site of the inoculation, about 5 cm. below the axilla, was sterilized by rubbing with cotton soaked with 95 per cent alcohol. Animals were not anaesthetized for the transplantation.

The weight of each animal was recorded every third or fourth day and the approximate size of its tumor was measured each

[1] The present writers wish to express their obligation to Dr. Wood, and also to Dr. Itami for his advice and suggestions concerning tumor inoculation.

week by means of calipers, and a drawing was made according to measurement. The final measurement and the weight of the grafted tumor were obtained at autopsy.

Tumor inoculation in animals fed upon the special diets and in control animals fed upon a normal diet was performed simultaneously.

The albino rats used were exclusively of our own laboratory breed from the stocks of a New York dealer and of Cornell Medical College. The normal diet consisted of wheat-bread soaked in whole milk, and fresh carrots or cabbage.

The animals, both males and females, selected for the comparative experiments were from 50 to 150 days old at the time of inoculation.

Fresh food was given each morning. The amount of food was not restricted throughout the series of experiments. Fresh tap water was given ad libitum.

The following special diets were employed. The methods of preparation for the individual food substances have been fully discussed in a previous article (28).

TABLE 1

Special banana diets

NAME OF SUBSTANCE	RATION A	RATION B	RATION C	RATION D
	A complete banana diet	A complete banana diet after lactation	Partially deficient in food accessory factors	Deficient in protein and partially deficient in food accessory factors
	per cent	*per cent*	*per cent*	*per cent*
Bananas	83.0	83.5	84.0	100
Casein	16.0	16.0	16.0	0.0
Yeast	0.5	0.5	0.0	0.0
Protein-free milk	0.5	0.0	0.0	0.0

A parabolic growth curve of the Flexner-Jobling rat carcinoma

During the past sixteen months we have made twenty successive transplantations using from 6 to 24 albino rats fed upon normal diet for each series, and have successfully grafted from 70 to 100 per cent of the animals, seldom as low as 50 per cent.

The development of these tumor grafts during the first few days was gradual and then continued very rapidly until the tumors broke down owing to their size and to injuries (Chart I). However, the rate of weight increase of the tumor is highest in the early stage of development, due to its rapid cell division, and falls rapidly until it reaches about the twenty-first day, and then very slowly declines. The curve of growth is smooth (Chart II).

The progressive growth of these tumors was obtained when the diet of the hosts was complete. We see from the data given in table 2 that the materials required for building up these new growths in the earlier days were small; and thus the body weights of the hosts remained unaltered.

TABLE 2

Showing the mean weight and dimensions of the Flexner-Jobling rat carcinoma on seven selected days

AGE IN DAYS	NUMBER OF TUMORS EXAMINED	AVERAGE WEIGHT OF TUMOR	AVERAGE SIZE OF TUMOR	RATE OF INCREASE IN WEIGHT
		gm.	*cmm.*	*per cent*
0	15	0.010	14.8	
7	11	0.113	196	1030
14	8	0.878	1280	677
21	10	3.10	4570	253
28	14	6.29	9040	103
35	17	10.8	16900	72
42	21	18.3	32200	70

We have noticed that the hosts generally remained in good health for nearly 42 days from the day of inoculation even though they slowly lost weight, possibly because the nutritive requirements of the tumor exceeded the food intake of the host and because the activity of the tumor disturbed the metabolism of the host.

Table 2 gives the average weight and dimensions of the tumors exclusive of cystic, local metastatic, and ulcerated tumors, obtained by killing and autopsying animals of a given age.

In Chart I we have shown the parabolic growth curve of the Flexner-Jobling rat carcinomata, the almost identical growth curve of fetuses (29), and also the linear growth of young albino rats during the period of lactation (28).

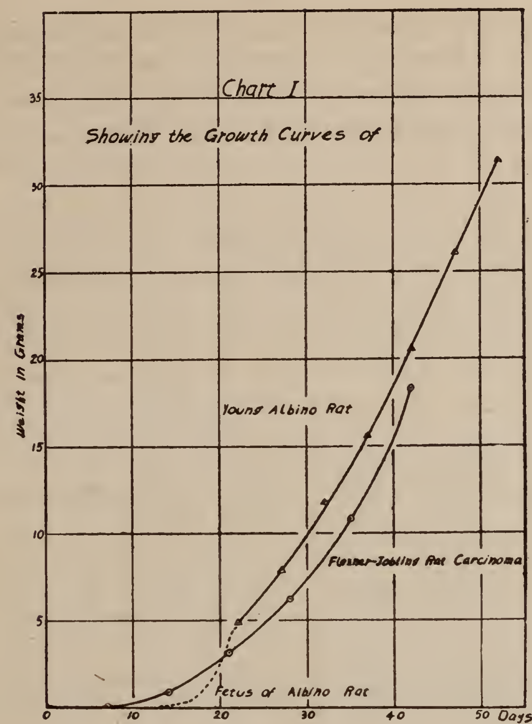


Chart I
Showing the Growth Curves of
Young Albino Rat
Flexner-Jobling Rat Carcinoma
Fetus of Albino Rat
Weight in Grams
35
30
25
20
15
10
5
0
10
20
30
40
50 Days

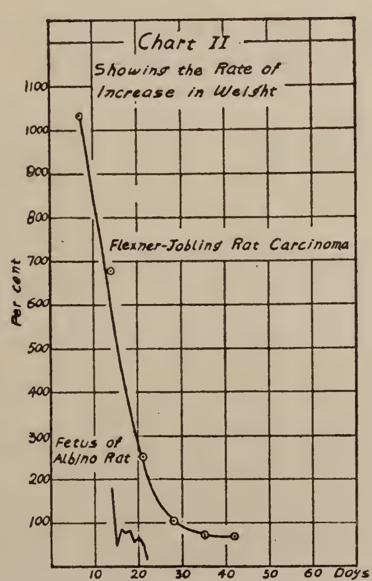
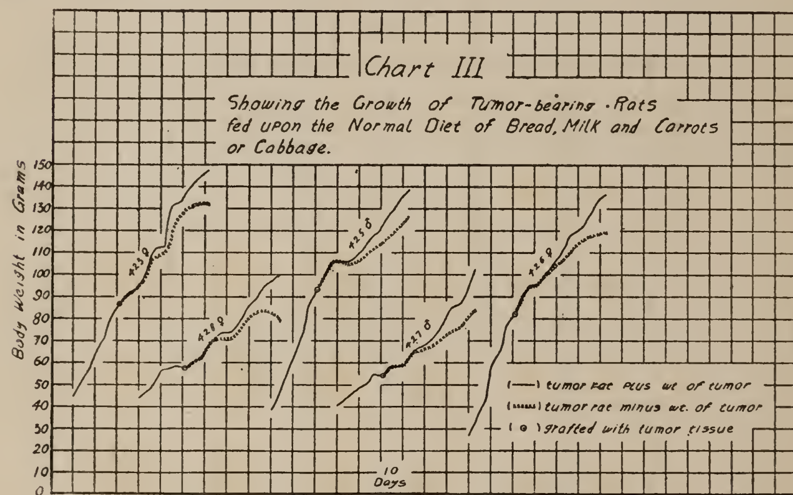


Chart II
Showing the Rate of
Increase in Weight
Flexner-Jobling Rat Carcinoma
Fetus of
Albino Rat
Per cent
1100
1000
900
800
700
600
500
400
300
200
100
10
20
30
40
50
60 Days

Chart III shows the growth curves of tumor-bearing rats fed upon a normal diet of bread, milk, and carrots or cabbage. The apparent weights of the tumors in each succeeding week except the final were made with the help of diagrams and surface measurements.



The influence of two complete diets, different in nature, upon tumor growth and susceptibility

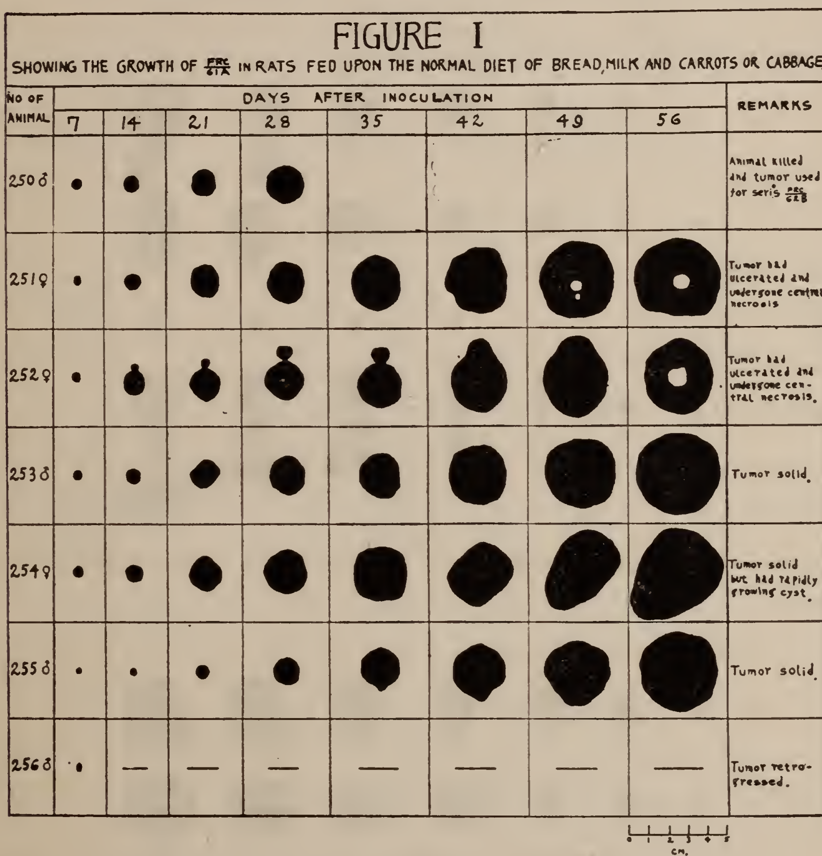
Experimental results by Osborne and Mendel, McCollum, and others, have shown that for very young growing animals the selection of the protein and food accessory substances is a matter of great importance, and also that the building up of their body tissues requires a relatively much greater amount of the growth promoting accessory factors than the adult animal requires.

It has been shown in a former article by the present writers that a diet consisting of bananas, 83 per cent, casein, 16 per cent, yeast, 0.5 per cent, and protein-free milk, 0.5 per cent is an adequate diet for the growth, maintenance, reproduction, and perfect milk production of the albino rat.

Experiment 1, September 18, 1918.

Eight young rats about two months old were placed on this

complete banana diet, seven control animals of a similar age and weight were fed upon the normal diet of bread, milk, and carrots or cabbage. Thirty-four days later tumor fragments were inoculated into these animals under aseptic precautions. The results of this experiment are shown in figures I and II.



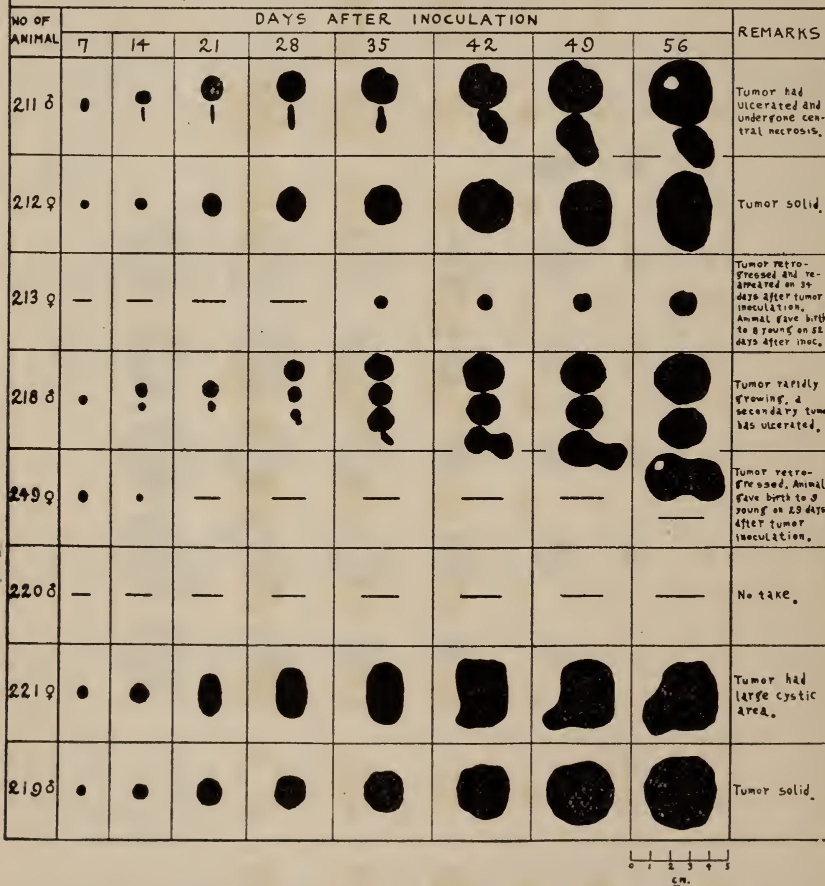
Experiment 2, November 19, 1918.

In order to eliminate differences in successful takes which might have resulted from the previous diet, we selected ten young rats from two litters of fourteen young which were successfully raised by mothers whose diet consisted of bananas, casein, yeast, and protein-free milk exclusively, and continued

feeding with the same diet before and after tumor inoculation. The average weight of the animals at the time of the tumor inoculation was 105 grams. A similar procedure was carried out on

FIGURE II

SHOWING THE GROWTH OF THE $\frac{FRC}{61A}$ IN RATS FED UPON A COMPLETE BANANA DIET OF BANANAS, 83.0; CASEIN, 16; YEAST 0.5; AND PROTEIN-FREE MILK, 0.5 PER CENT.



those control animals fed on a normal diet. The results of this experiment are shown in figures III and IV.

These two experiments show very clearly that the growth of, and susceptibility to the tumor in rats is not altered by the difference in the *character* of the food given in these experiments.

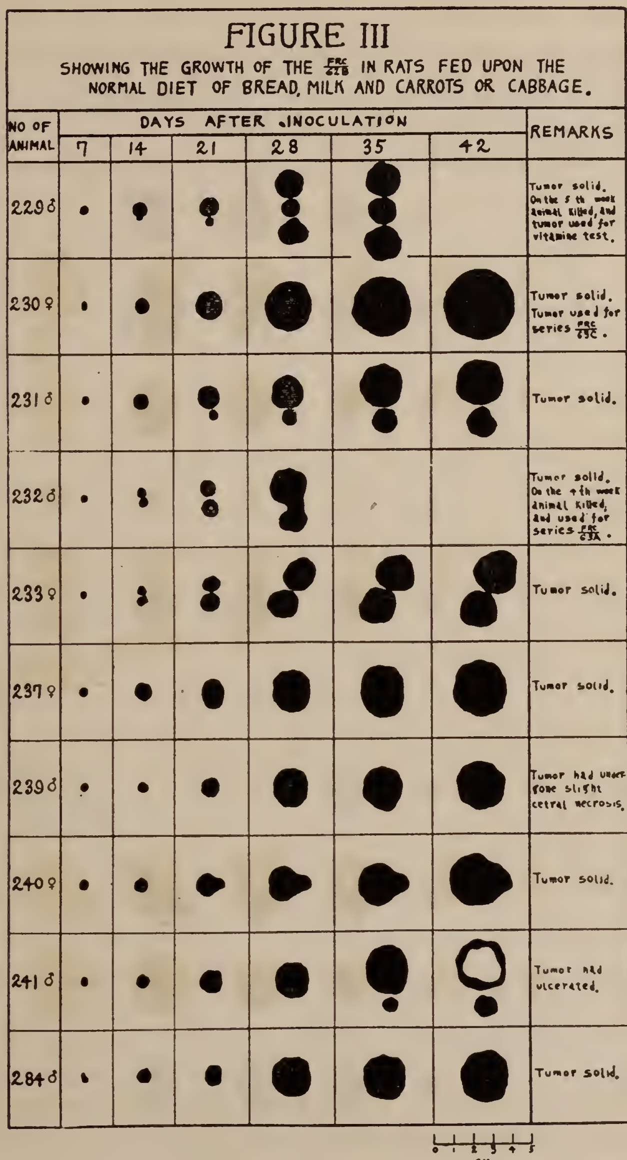


FIGURE III
SHOWING THE GROWTH OF THE FRC/62B IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.
NO OF ANIMAL
DAYS AFTER INOCULATION
7
14
21
28
35
42
REMARKS
229 ♂
Tumor solid. On the 5 th week animal killed, and tumor used for vitamine test.
230 ♀
Tumor solid. Tumor used for series FRC/63C.
231 ♂
Tumor solid.
232 ♂
Tumor solid. On the 4 th week animal killed, and used for series FRC/63A.
233 ♀
Tumor solid.
237 ♀
Tumor solid.
239 ♂
Tumor had undergone slight cetral necrosis.
240 ♀
Tumor solid.
241 ♂
Tumor had ulcerated.
284 ♂
Tumor solid.
0 1 2 3 4 5
CM

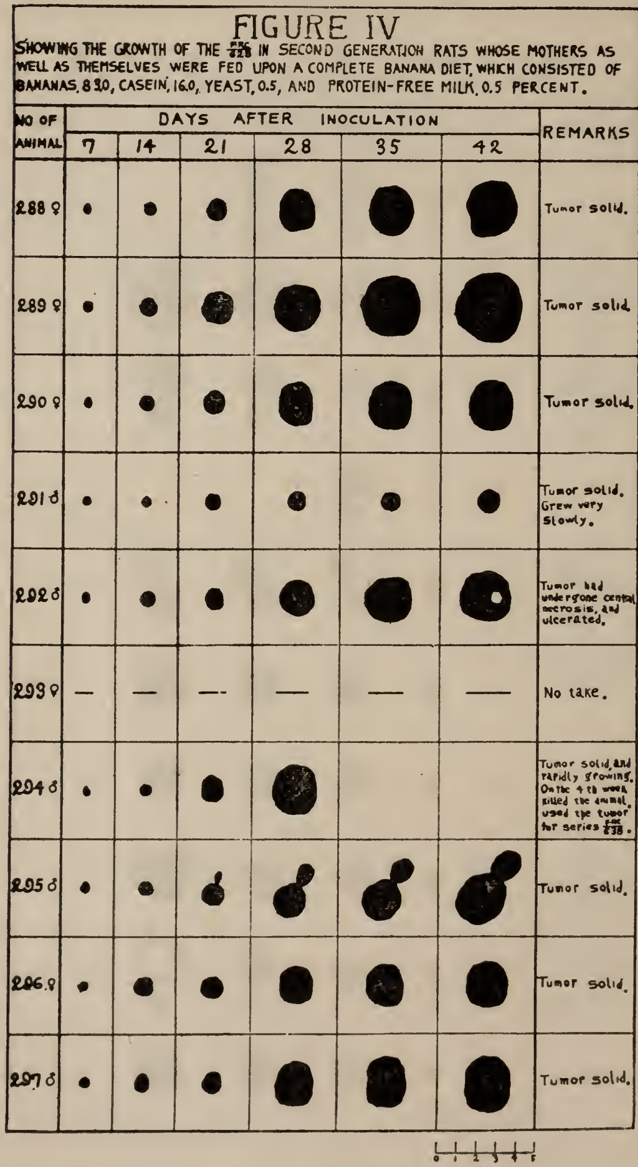


FIGURE IV
SHOWING THE GROWTH OF THE IN SECOND GENERATION RATS WHOSE MOTHERS AS WELL AS THEMSELVES WERE FED UPON A COMPLETE BANANA DIET, WHICH CONSISTED OF BANANAS, 83.0, CASEIN, 16.0, YEAST, 0.5, AND PROTEIN-FREE MILK, 0.5 PERCENT.
NO OF ANIMAL
DAYS AFTER INOCULATION
7
14
21
28
35
42
REMARKS
288 ♀
Tumor solid.
289 ♀
Tumor solid.
290 ♀
Tumor solid.
291 ♂
Tumor solid. Grew very slowly.
292 ♂
Tumor had undergone central necrosis, and ulcerated.
293 ♀
No take.
294 ♂
Tumor solid, and rapidly growing. On the 4th week killed the animal, used the tumor for series
295 ♂
Tumor solid.
296 ♀
Tumor solid.
297 ♂
Tumor solid.
0 1 2 3 4 5
CM.

We have shown that a mixture of bananas, casein, and yeast provided all the food requirements of young rats for normal growth and reproduction (26). Such a diet is, however, deficient in some substance essential to the production of proper milk by the mother. In the next experiment the effect of this slightly deficient diet upon the growth of tumors was studied.

Experiment 3, April 3, 1919.

Twenty young healthy rats were divided into two groups. Ten of them were placed on a special diet of bananas, 83.5 per cent, casein, 16 per cent, and yeast, 0.5 per cent, while the other ten were fed upon the normal diet. Fourteen days later tumor fragments were inoculated into all these animals under aseptic precautions.

The results of this experiment are shown in figures V and VI.

According to figures V and VI the development of the tumor grafts in the animals fed upon a complete banana diet was somewhat retarded. Most of these tumor-bearing animals showed subnormal growth throughout the experimental period. At the end of the sixth week it was found that the average weight of the tumors was 6.1 grams. On the other hand, the control animals, fed upon bread, milk, and carrots or cabbage, increased rapidly in weight, as we have shown in Chart III. The average weight of the tumors was 16.4 grams, or more than two and a half times that of the specially fed animals.

We may conclude, however, that the retarded growth of the tumors was probably due to the influence of the subnormal growth of the hosts in this particular experiment.

In the next experiment we made use of a diet more decidedly restricted in accessory food substances. This diet consisted of bananas, 84 per cent, and purified casein, 16 per cent. We have previously shown that such a diet is inadequate for the normal growth of young rats (26).

Experiment 4, December 18, 1918.

Seven young rats, the average weight of which was 97 grams, were fed with this special diet for two days prior to the tumor inoculation. Eight animals on normal diet were used as controls.

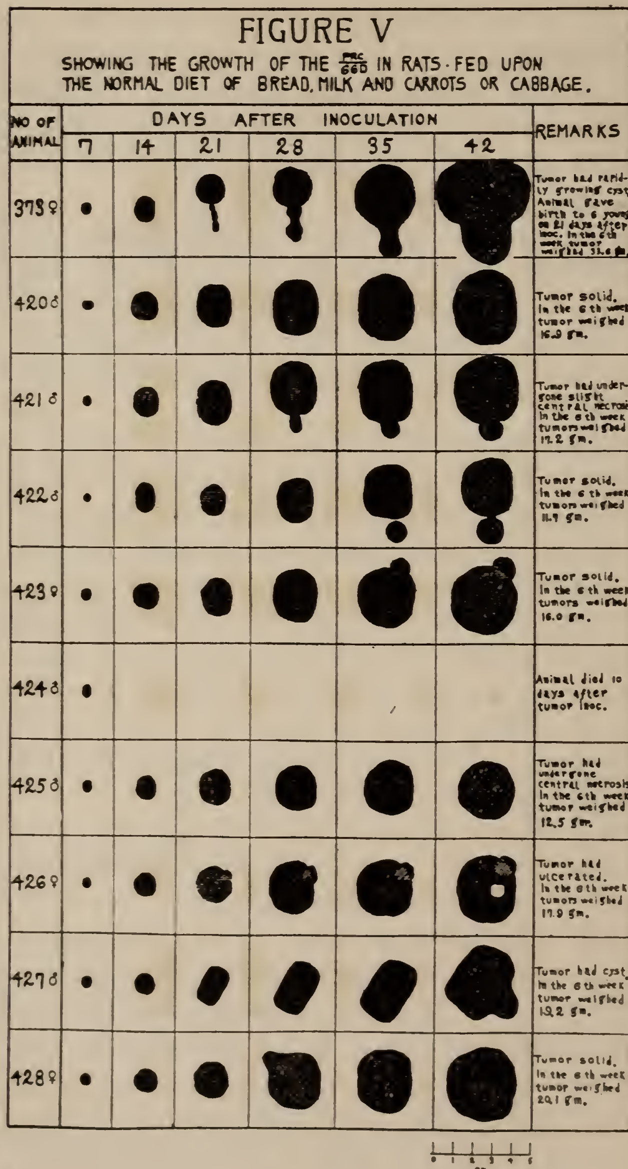


FIGURE V
SHOWING THE GROWTH OF THE $\frac{PRC}{660}$ IN RATS·FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.
NO OF ANIMAL
DAYS AFTER INOCULATION
7
14
21
28
35
42
REMARKS
378 ♀
Tumor had rapidly growing cyst. Animal gave birth to 6 young on 21 days after inoc. In the 6th week tumor weighed
420 ♂
Tumor solid. In the 6 th week tumor weighed 16.9 gm.
421 ♂
Tumor had undergone slight central necrosis. In the 6 th week tumors weighed 17.2 gm.
422 ♂
Tumor solid. In the 6 th week tumors weighed 11.9 gm.
423 ♀
Tumor solid. In the 6 th week tumors weighed 16.0 gm.
424 ♂
Animal died 10 days after tumor inoc.
425 ♂
Tumor had undergone central necrosis. In the 6th week tumor weighed 12.5 gm.
426 ♀
Tumor had ulcerated. In the 6th week tumors weighed 17.9 gm.
427 ♂
Tumor had cyst. In the 6 th week tumor weighed
428 ♀
Tumor solid. In the 6 th week tumor weighed 20.1 gm.
CM.

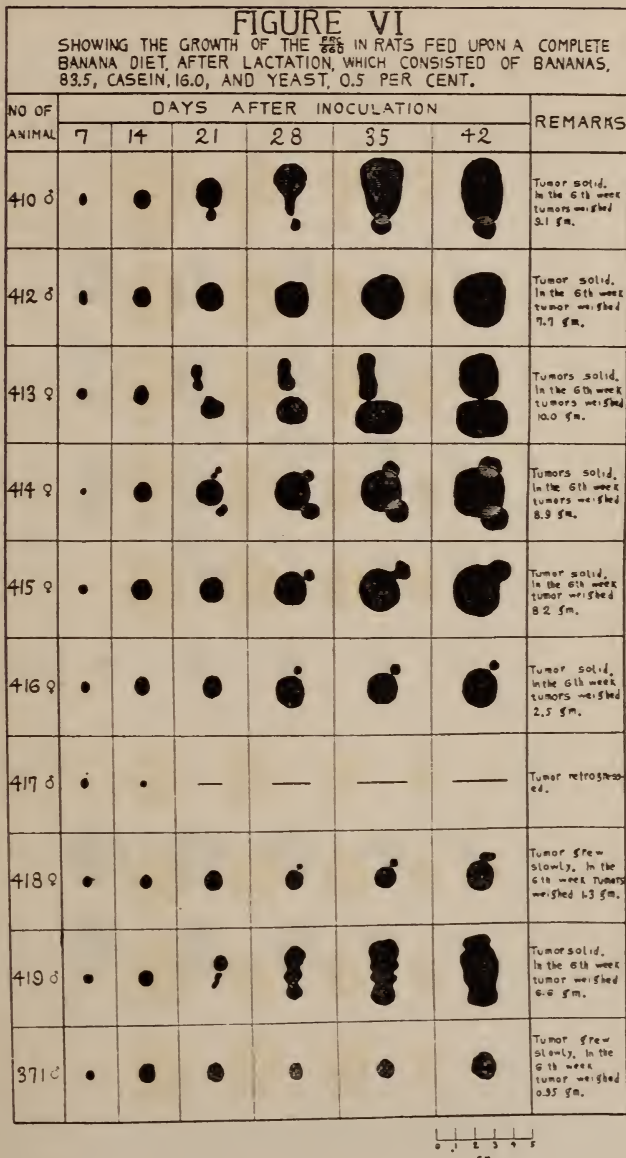
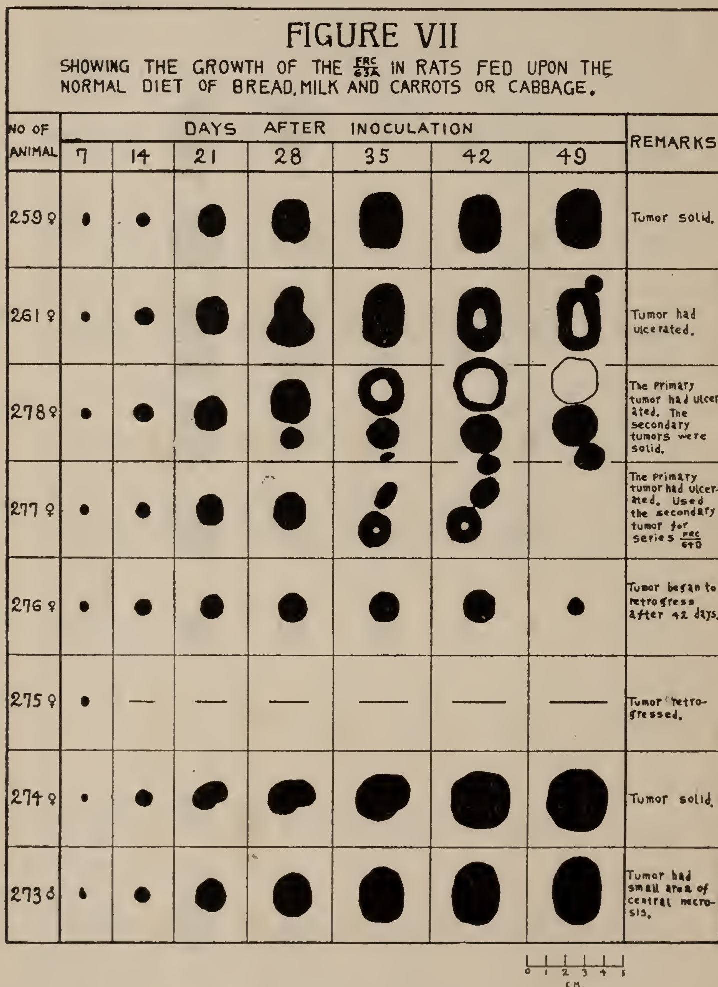


FIGURE VI
SHOWING THE GROWTH OF THE FRC 660 IN RATS FED UPON A COMPLETE BANANA DIET, AFTER LACTATION, WHICH CONSISTED OF BANANAS, 83.5, CASEIN, 16.0, AND YEAST, 0.5 PER CENT.
NO OF ANIMAL
DAYS AFTER INOCULATION
7
14
21
28
35
42
REMARKS
410 ♂
Tumor solid. In the 6th week tumors weighed 9.1 gm.
412 ♂
Tumor solid. In the 6th week tumor weighed 7.7 gm.
413 ♀
Tumors solid. In the 6th week tumors weighed 10.0 gm.
414 ♀
Tumors solid. In the 6th week tumors weighed 8.9 gm.
415 ♀
Tumor solid. In the 6th week tumor weighed 8 2 gm.
416 ♀
Tumor solid. In the 6th week tumors weighed 2.5 gm.
417 ♂
Tumor retrogressed.
418 ♀
Tumor grew slowly. In the 6th week Tumors weighed 1.3 gm.
419 ♂
Tumor solid. In the 6th week tumor weighed 6.6 gm.
371 ♂
Tumor grew slowly. In the 6th week tumor weighed 0.95 gm.
0 1 2 3 4 5
CM.

The results obtained from this experiment are shown in figures VII and VIII.



From these results it would be concluded that the banana-casein diet has a decidedly retarding influence upon tumor growth and an inhibitory action upon tumor susceptibility. This single experiment, however, does not warrant us in drawing a definite conclusion.

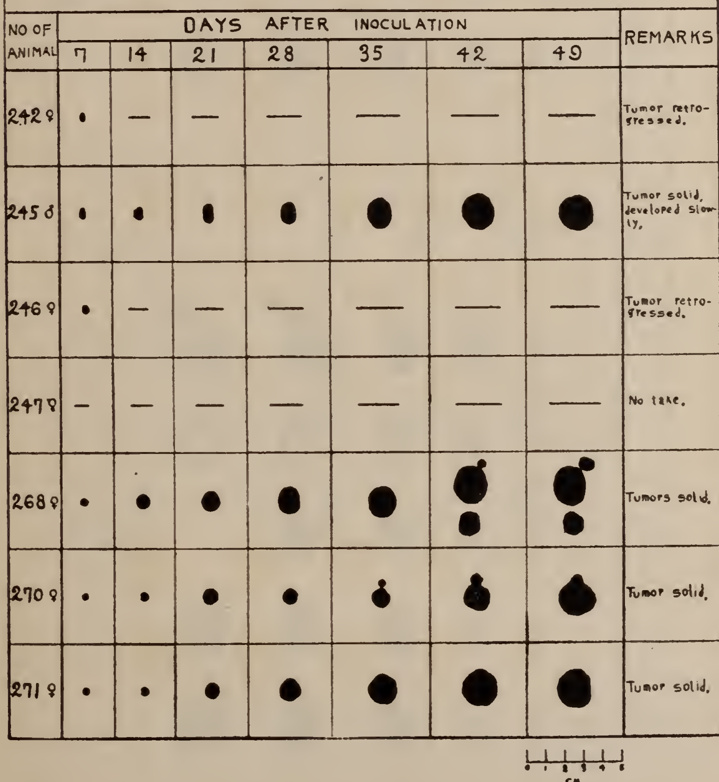
The experiment was repeated with nodules of $\frac{FRC}{67E}$.

Experiment 5, May 22, 1919.

Four young rats were placed on the banana-casein diet and eight young rats we continued feeding on our normal diet. Fif-

FIGURE VIII

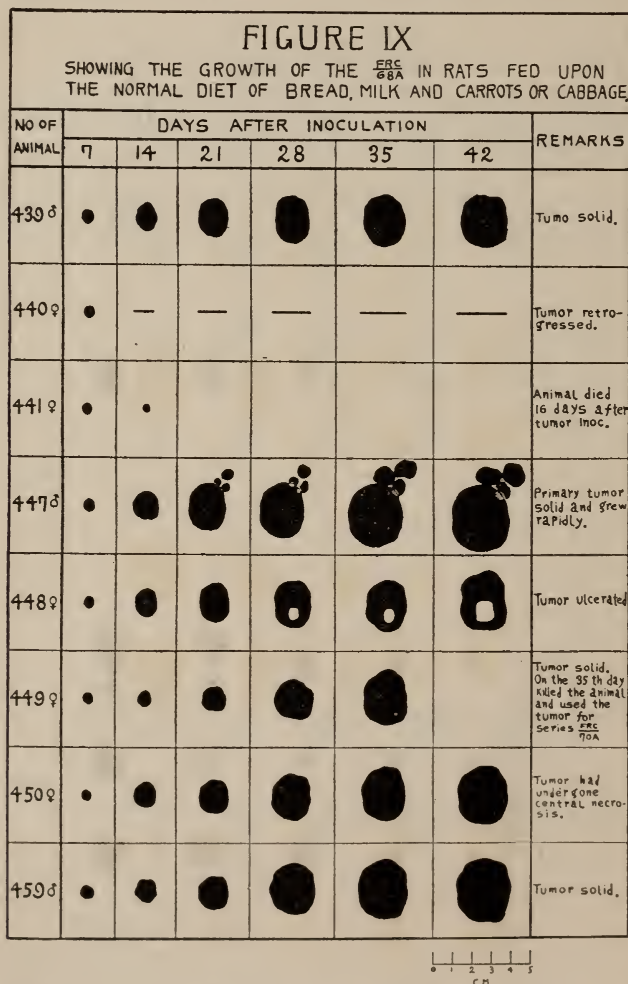
SHOWING THE GROWTH OF THE $\frac{FRC}{63A}$ IN RATS FED UPON A DIET PARTIAL DEFICIENT IN FOOD ACCESSORY FACTORS CONSISTING OF BANANAS, 84, AND CASEIN, 16 PER CENT.



teen days later tumor bits were inoculated into these animals under aseptic precautions.

The results obtained from this experiment are recorded in figures IX and X.

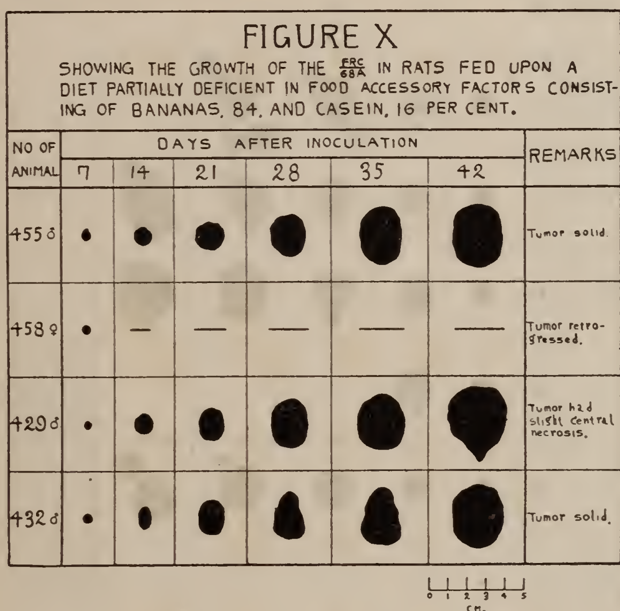
It is seen that the grafted tumors grew with equal rapidity in hosts fed upon the banana-casein diet and in the controls. A difference, therefore, may exist among tumors of different generations in the nutriment required for their development.



In an earlier paper (26) we have shown that young albino rats have failed to maintain body weight upon an exclusive banana diet. Animals either remained at nearly constant body weight after the first day's drop, or kept losing weight steadily until

death. Many animals lived upon bananas for nearly two and a half months, but did not grow. We stated that the banana is deficient in (a) protein, and (b) the water-soluble accessory factor, because the addition of definite amounts of casein and yeast to the banana resulted in rapid growth of young rats.

Three sets of experiments were made to determine the effect of bananas alone upon the growth of and susceptibility to tumor, in the following manner:



Experiment 6, February 15, 1919.

Twenty young healthy rats, the average weight of which was 78 grams, were divided into two groups. Ten of them were placed on the banana diet and the remaining ten were kept on the normal diet. Fourteen days later tumor fragments were inoculated into these animals under aseptic precautions. The results obtained from this experiment are shown in figures XI and XII.

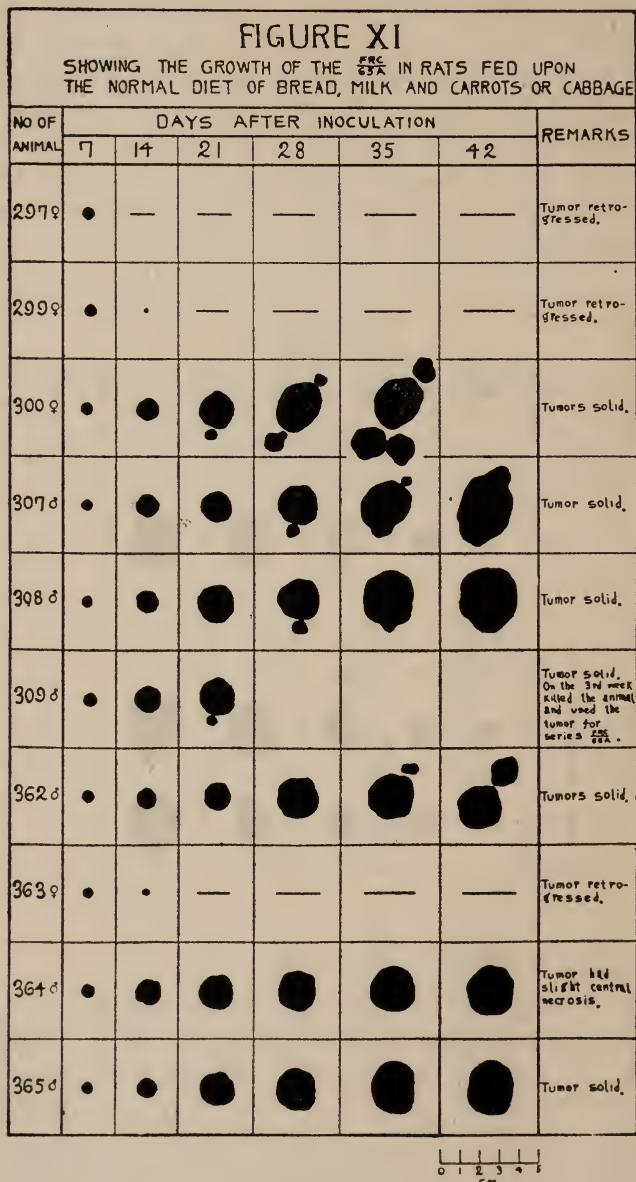


FIGURE XI
SHOWING THE GROWTH OF THE FRC/63A IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE
NO OF ANIMAL
DAYS AFTER INOCULATION
7
14
21
28
35
42
REMARKS
297♀
Tumor retrogressed.
299♀
Tumor retrogressed.
300♀
Tumors solid.
307♂
Tumor solid.
308♂
Tumor solid.
309♂
Tumor solid. On the 3rd week killed the animal and used the tumor for series
362♂
Tumors solid.
363♀
Tumor retrogressed.
364♂
Tumor had slight central necrosis.
365♂
Tumor solid.
0 1 2 3 4 5
CM.

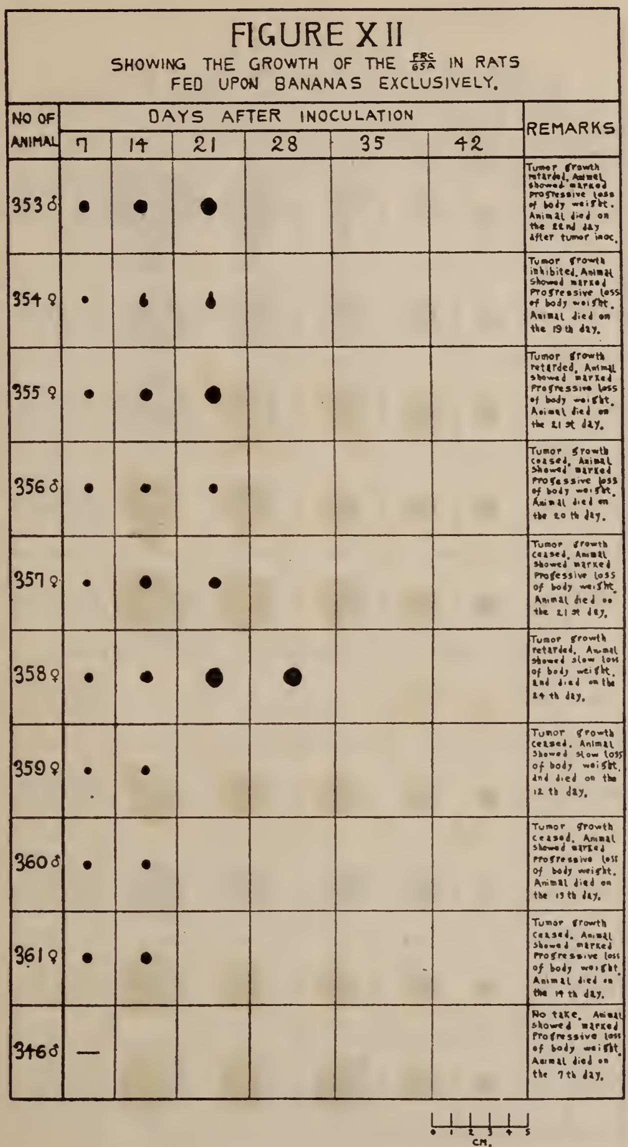


FIGURE XII
SHOWING THE GROWTH OF THE FRC 65A IN RATS FED UPON BANANAS EXCLUSIVELY.
NO OF ANIMAL
DAYS AFTER INOCULATION
7
14
21
28
35
42
REMARKS
353 ♂
Tumor growth retarded. Animal showed marked progressive loss of body weight. Animal died on the 22nd day after tumor inoc.
354 ♀
Tumor growth inhibited. Animal showed marked progressive loss of body weight. Animal died on the 19th day.
355 ♀
Tumor growth retarded. Animal showed marked progressive loss of body weight. Animal died on the 21st day.
356 ♂
Tumor growth ceased. Animal showed marked progessive loss of body weight. Animal died on the 20th day.
357 ♀
Tumor growth ceased. Animal showed marked progessive loss of body weight. Animal died on the 21st day.
358 ♀
Tumor growth retarded. Animal showed slow loss of body weight, and died on the 24th day.
359 ♀
Tumor growth ceased. Animal showed slow loss of body weight, and died on the 12th day.
360 ♂
Tumor growth ceased. Animal showed marked progressive loss of body weight. Animal died on the 13th day.
361 ♀
Tumor growth ceased. Animal showed marked progressive loss of body weight. Animal died on the 14th day.
346 ♂
No take. Animal showed marked progressive loss of body weight. Animal died on the 7th day.
0 1 2 3 4 5
CM.

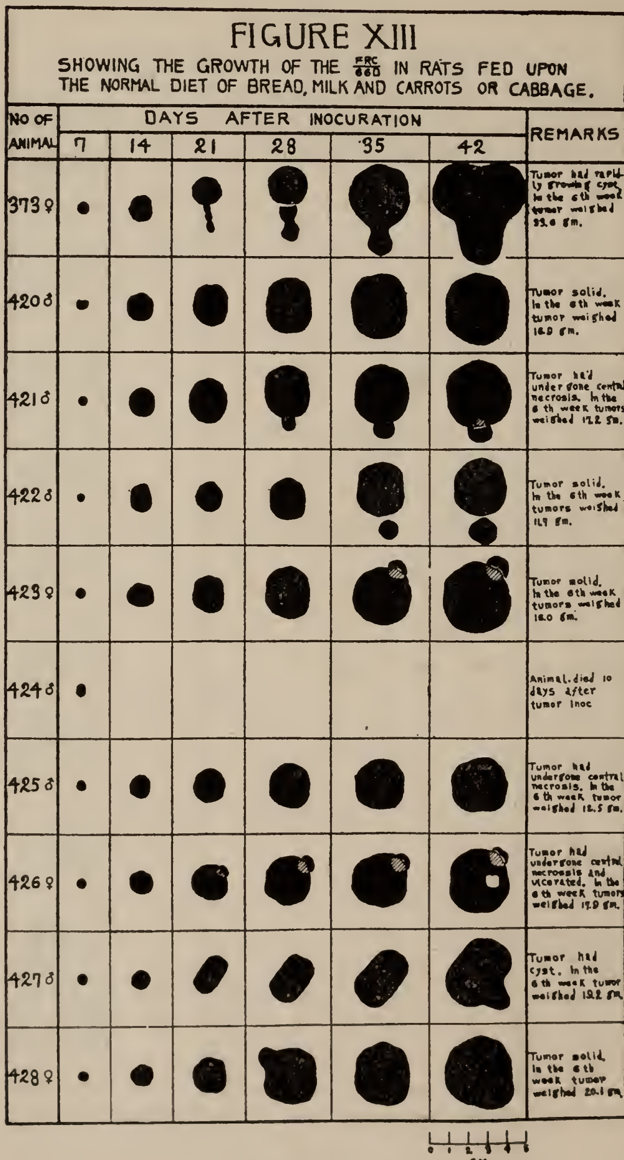
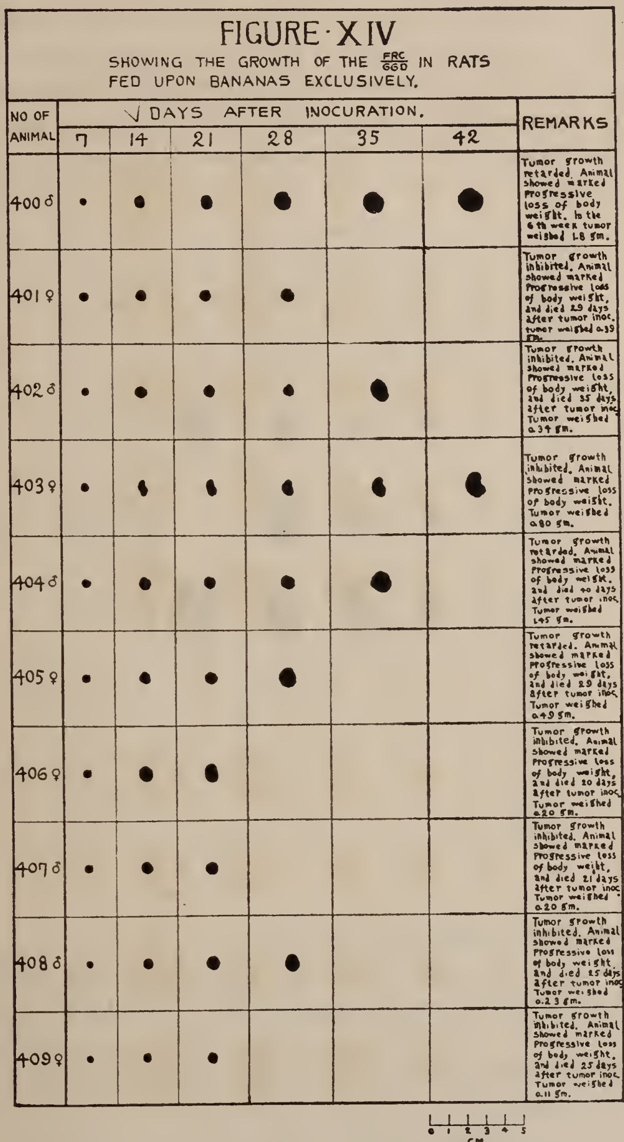
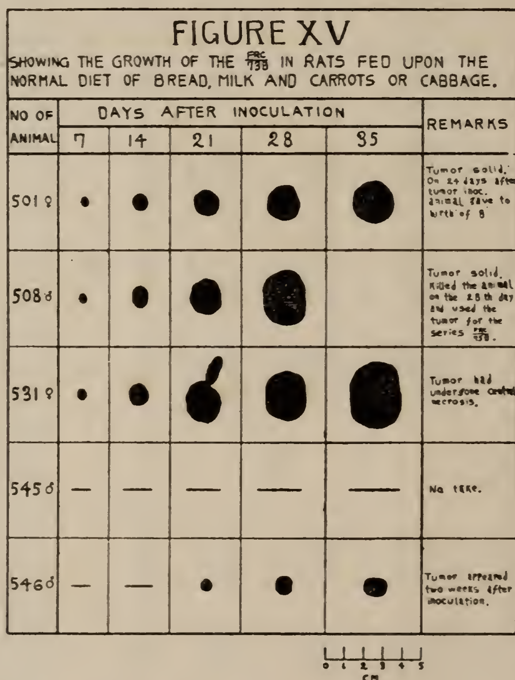


FIGURE XIII
SHOWING THE GROWTH OF THE FRC/66D IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.
NO OF ANIMAL
DAYS AFTER INOCURATION
7
14
21
28
35
42
REMARKS
373 ♀
Tumor had rapidly growing cyst. In the 6th week tumor weighed 33.0 gm.
420 ♂
Tumor solid. In the 6th week tumor weighed 16.0 gm.
421 ♂
Tumor had undergone central necrosis. In the 6th week tumors weighed 12.2 gm.
422 ♂
Tumor solid. In the 6th week tumors weighed 11.9 gm.
423 ♀
Tumor solid. In the 6th week tumors weighed 16.0 gm.
424 ♂
Animal died 10 days after tumor inoc
425 ♂
Tumor had undergone central necrosis. In the 6th week tumor weighed 12.5 gm.
426 ♀
Tumor had undergone central necrosis and ulcerated. In the 6th week tumors weighed 17.0 gm.
427 ♂
Tumor had cyst. In the 6th week tumor weighed 12.2 gm.
428 ♀
Tumor solid. In the 6th week tumor weighed 20.1 gm.
CM.


FIGURE·XIV
SHOWING THE GROWTH OF THE FRC/66D IN RATS FED UPON BANANAS EXCLUSIVELY.
NO OF ANIMAL
DAYS AFTER INOCURATION.
7
14
21
28
35
42
REMARKS
400 ♂
Tumor growth retarded. Animal showed marked progressive loss of body weight. In the 6th week tumor weighed 1.8 gm.
401 ♀
Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 29 days after tumor inoc. tumor weighed 0.39 gm.
402 ♂
Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 35 days after tumor inoc Tumor weighed 0.34 gm.
403 ♀
Tumor growth inhibited. Animal showed marked progressive loss of body weight. Tumor weighed 0.80 gm.
404 ♂
Tumor growth retarded. Animal showed marked progressive loss of body weight. and died 40 days after tumor inoc Tumor weighed 1.45 gm.
405 ♀
Tumor growth retarded. Animal showed marked progressive loss of body weight, and died 29 days after tumor inoc Tumor weighed 0.49 gm.
406 ♀
Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 20 days after tumor inoc Tumor weighed 0.20 gm.
407 ♂
Tumor growth inhibited. Animal showed marked progressive loss of body weight, and died 21 days after tumor inoc Tumor weighed 0.20 gm.
408 ♂
Tumor growth inhibited. Animal showed marked progressive loss of body weight. and died 25 days after tumor inoc Tumor weighed 0.23 gm.
409 ♀
Tumor growth inhibited. Animal showed marked progressive loss of body weight. and died 25 days after tumor inoc. Tumor weighed 0.11 gm.
0 1 2 3 4 5
CM.

Experiment 7, April 8, 1919.

Ten rats, ranging from 59 to 133 grams, were placed on the banana diet fourteen days prior to tumor inoculation, and the diet continued. Ten controls, ranging from 50 to 139 grams, were fed on the normal diet before and after the tumor inoculation. The results obtained from this experiment are shown in figures XIII and XIV.



Experiment 8, September 15, 1919.

Four of nine vigorous rats were changed to the banana diet from the normal diet seven days before the tumor inoculation, while the other five animals were allowed to remain on the normal diet. The results obtained from this experiment are shown in figures XV and XVI.

From these experiments it is plain that the banana diet had a marked retarding action upon the growth of the tumor grafts. The presence of tumor fragments in banana-fed animals hastened their death. The retarding influence of the banana diet

upon transplanted tumors is less marked if the normal diet of the animals is changed to a banana diet within seven days previous to the inoculation. The banana diet has no specific influence upon tumor susceptibility.

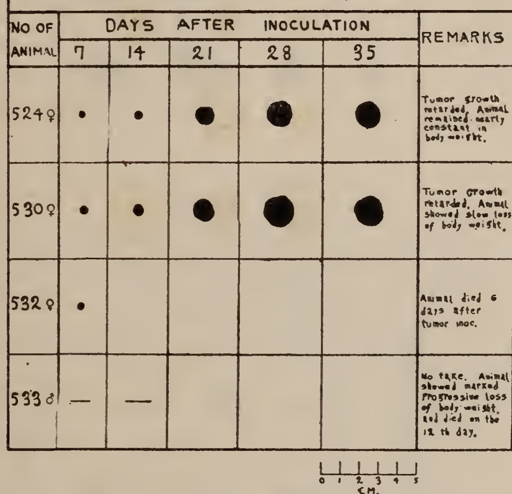
Further proof of the inhibitory action of bananas upon the growth of tumor grafts is shown in the following two experiments.

Experiment 9, May 19, 1919.

In the first experiment the normal diet of tumor-bearing animals was changed to an exclusive banana diet at the end of the

FIGURE XVI

SHOWING THE GROWTH OF THE IN RATS FED UPON BANANAS EXCLUSIVELY.



fourteenth day after tumor inoculation, the time when grafted tumors begin to develop more rapidly (see Chart I). The results from this experiment are given in figures XVII and XVIII.

Experiment 10, September 18, 1919.

The second experiment was just the reverse of the preceding. Five of nine tumor-bearing animals fed upon exclusive banana diet were given normal diet fourteen days after tumor inoculation. The results are shown in figures XIX and XX.

FIGURE XVII

SHOWING THE GROWTH OF THE $\frac{FRC}{67E}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.

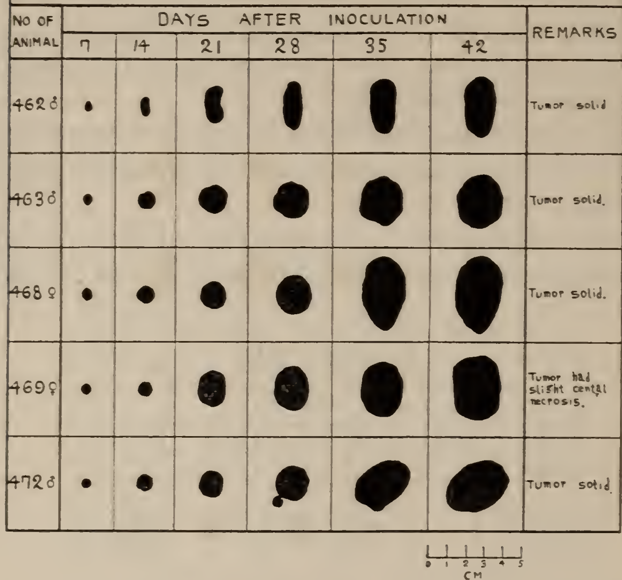
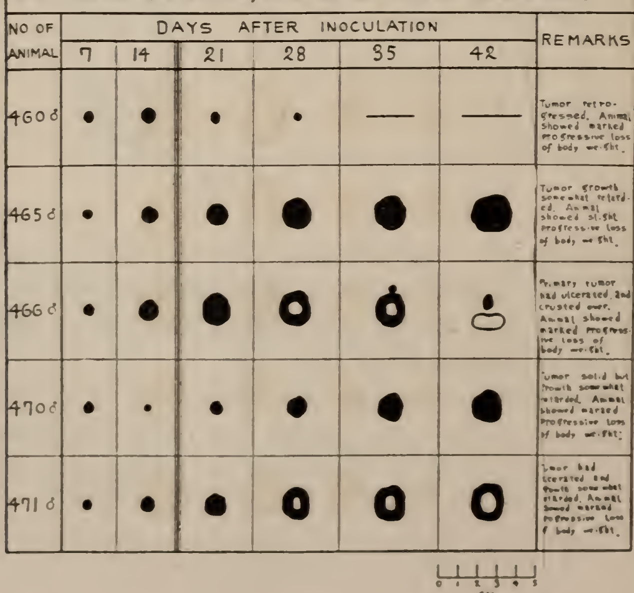


FIGURE XVIII

SHOWING THE GROWTH OF THE $\frac{FRC}{67E}$ IN RATS FED UPON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE UNTIL TWO WEEKS AFTER TUMOR INOCULATION, THEN ON AN EXCLUSIVE BANANA DIET.



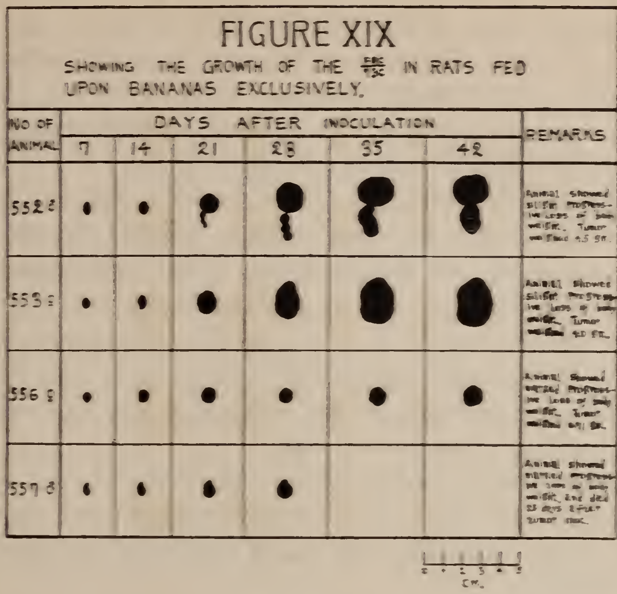


FIGURE XIX

SHOWING THE GROWTH OF THE [illegible] IN RATS FED UPON BANANAS EXCLUSIVELY.

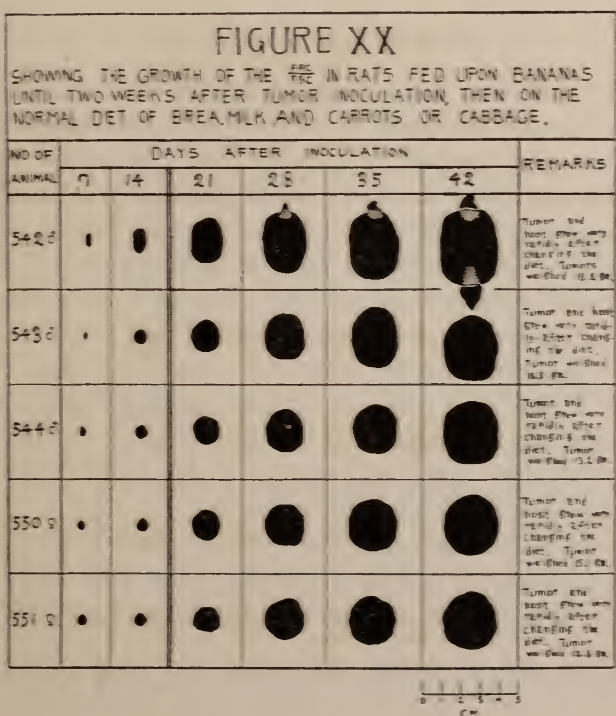


FIGURE XX

SHOWING THE GROWTH OF THE [illegible] IN RATS FED UPON BANANAS UNTIL TWO WEEKS AFTER TUMOR INOCULATION, THEN ON THE NORMAL DIET OF BREAD, MILK AND CARROTS OR CABBAGE.

Rats 460, 465, 466, 470, and 471 of the first part of the present experiments lost steadily in body weight when their normal diet was changed to an exclusive banana diet. Their tumors grew, but not at the normal rate. It appears that the tissues of the hosts supplied nutrient material to the tumors.

Rats 542, 543, 544, 550, and 551 of the second part of the present investigations grew supernormally for the first few days after they were returned to a complete diet. Also the development of the tumor nodules was very rapid and reached to the normal sizes for the third, fourth, fifth, and sixth week respectively.

We have successfully inoculated tissues of a stunted tumor taken from a rat fed upon exclusive banana diet into young rats that were feeding upon normal diet.

These experiments give evidence that our diets did not alter the malignant character of the Flexner-Jobling rat carcinoma, though in some instances the diets markedly diminished the rate of growth.

HISTOLOGICAL OBSERVATIONS

Dr. James Ewing has kindly examined microscopically our tumor specimens[2] obtained from rats fed upon normal diet of bread, milk, and carrots or cabbage, and those fed upon our special banana diets, namely, banana-casein-yeast-protein-free milk; banana-casein-yeast; banana-casein; and banana alone.

He has drawn the following general conclusions:

1. General structure of tumors from rats fed upon normal diet and a complete banana diet is identical.

2. The stunted tumors taken from rats fed upon exclusive banana diet show the presence of well nourished cells, free from areas of necrosis and generally free from fibrosis, indicating full vitality. In other words, the tumors of banana-fed animals are as well nourished as those of the controls.

It is interesting to note that these histological findings corroborate the experimental results in showing that the malignant character of the growth was not changed by the banana diet.

[2] The writers wish to express their appreciation to Mrs. A. Punshon of Memorial Hospital, who has kindly prepared the histological material.

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